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News&Features from NIH

January 1982

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

NIH PHOTO 1743

News&Features from NIH

January 1982

Hybridomas, one of the most promising biotechnologies of the future, have already allowed researchers to explore some fundamental aspects of immunity. At a recent NIH science writers seminar, prominent investigators expressed the hope that this powerful research tool will enable them to manipulate the immune system, ultimately aiding in the diagnosis and treatment of disease. See page 4.

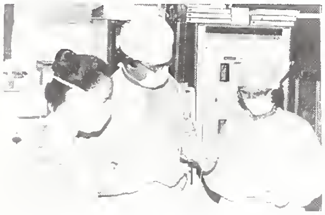
CT scanning of the brain for brain tumors, hemorrhages, and infections, is efficient, safe, and very accurate, according to members of a panel who recently met at the NIH for a Consensus Development conference.

However, the panel cautioned that the CT scanner should be used as a diagnostic tool for complex brain disorders and not for minor head trauma or headaches. See page 6.

In April 1979, the NIH's research hospital opened their new Intensive Care Unit (ICU), located on the 10th floor of the Clinical Center (CC).

The unit is designed to care for CC patients who require critical intensive care for several days.

The ICU also provides surgical intensive care for patients from the Cancer and Heart surgical units, since many of these patients require special care in dealing with their multi-organ system dysfunctions. See page 2.



Meningococcal meningitis is one of the more common causes of meningitis in children under the age of two.

Until now, there has not been an effective vaccine to protect these youngsters. However, Baylor College of Medicine in Houston has just completed initial testing in adults for a vaccine which seems to prevent the group B meningococcal organism.

Results so far show that most of the volunteers showed a significant antibody response. This vaccine, say the researchers, stimulates antibody production in adults. However further testing must be completed before youngsters can receive the vaccine as part of their inoculation program. See page 7.

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Editorial Staff

Mary Donovan
Editor
Susan Gerhold
Associate Editor
Helen Ou
Special Assistant
Maureen Napper
Editorial Assistant

NIH News Branch
Building 31, Room 2B-10
Bethesda, Maryland 20205
Phone:
(301) 496-2535

Care of the Critically Ill: An Emerging Medical Subspecialty

Marian Segal

On a Sunday afternoon several weeks ago a young man participating in an NIH study on Hodgkin's disease entered the Clinical Center seriously ill with pneumonia. Despite treatment, his condition steadily worsened during the course of the day. He became short of breath, his blood pressure dropped precipitously, and within a few hours' time he was barely conscious. That evening he was transferred to the intensive care unit on 10D where he was immediately put on a ventilator to assist his breathing, given fluids intravenously, and administered medication to raise his blood pressure. With constant surveillance and intensive treatment, his condition improved dramatically over the next few hours, and by Monday morning he was awake and alert. Tuesday he was able to breathe without mechanical assistance, and two weeks later he was well enough for discharge.

Since the CC's ICU opened in April 1979, patients with a wide variety of underlying clinical problems have been admitted for management of a severe problem they all share—major organ system failure requiring critical care support.

In recent years, care of the critically ill has evolved into an important new medical subspecialty. "The field has emerged with the changing role of hospitals," explains Dr. Joseph Parrillo, chief of the Critical Care Medicine Department (CCMD). "As more and more mild-to-moderately ill patients are being well cared for outside the hospital environment, hospitals are evolving into centers for the most severely ill, and, in response to this trend, medical centers worldwide are establishing departments of critical care medicine."

The Clinical Center instituted the CCMD with a threefold mission: to manage intensely ill patients in the intensive care unit and provide critical care consultations throughout the hospital; to educate physicians and staff on optimal management of these patients; and to conduct research in the field. The department operates the 10D ICU, which serves as the medical intensive care unit for the hospital and also provides surgical intensive care for postoperative patients referred from the heart and cancer surgical services. Because some post-surgery patients have multi-organ system dysfunction, they may be transferred to this unit for special care. In addition, patients in clinical protocols requiring hemodyanic monitoring or involving potential life-threatening risks are cared for in the ICU.

Nurse Dorothy Czerski (l), Dr. Joseph Parrillo (c), and Dr. Margaret Parker (r) prepare a Swan-Ganz catheter for insertion into a patient to help manage blood pressure.

NIH PHOTO 1744

Intensive care requires special equipment and special personnel. Professional and support staff receive training in the complex problems and needs of critically ill patients to give them the best chance for survival. The ICU is staffed by a cardiologist, pulmonary physician, and an anesthesiologist specially trained in critical care medicine. Because the nature of critical illness involves several major organ systems, the ICU staff receives support from Institute physicians specializing in infectious disease, kidney disorders, and gastrointestinal malfunction.

"The ICU environment and patient population demand a specially trained support staff as well," says Dr. Parrillo. "Nurses must be able to manage unconscious patients on respirators, perform cardiac resuscitation, and respond quickly to sudden changes in patient status." Technicians in the unit also perform more diverse functions than those in most other hospital areas. They operate and monitor highly sophisticated computer equipment and must learn to run a number of diagnostic tests normally done by several technicians versed in separate procedures.

Critically ill patients require constant minute-to-minute assessment of clinical, hemodyamic, and laboratory data. To aid in this evaluation, the ICU is equipped with sophisticated computer systems that collect, sort, store, and analyze vast quantities of patient data. Measurements of arterial pressures, cardiac output, pulmonary artery pressure, blood gases, and other vital functions are provided immediately and continuously, permitting constant assessment of each patient's status. This capability is essential in caring for very sick people whose condition can change drastically within moments, especially in response to drug administration or other therapeutic manipulation. The physician also uses the stored information to evaluate treatment and guide course of therapy.

Most ICU patients are on a respirator or other sophisticated life support systems, and moving them out of the unit for diagnostic studies could jeopardize their care or even threaten life. To minimize this danger, Dr. Parrillo is setting up facilities for heart catheterizations, echocardiography, and radionuclide studies of heart function and coronary artery blood flow to be done in the unit itself. Patients undergo only those tests that will provide specific information needed to guide therapy at minimum risk to the patient. The diagnostic equipment and computer capabilites enable physicians to evaluate changes in cardiovascular size, shape, and function in acute illness, discover specific areas of malfunction, and develop rational modes of therapy.

Because critical care medicine is a new discipline, most research thus far has centered on developing and refining life support technology. The Clinical Center's CCMD plans to concentrate on studies of the causes and underlying mechanisms of critical illness—a relatively unexplored area in medicine. The monitoring equipment and computer facilities in the ICU permit detailed clinical studies in critically ill patients, which will be supported by basic research on the biochemical and immunological factors involved in hemodynamic and pulmonary dysfunction. ■

Virus Causes Diabetes in Mice and Triggers Antibodies to Normal Tissues

Hilah Thomas

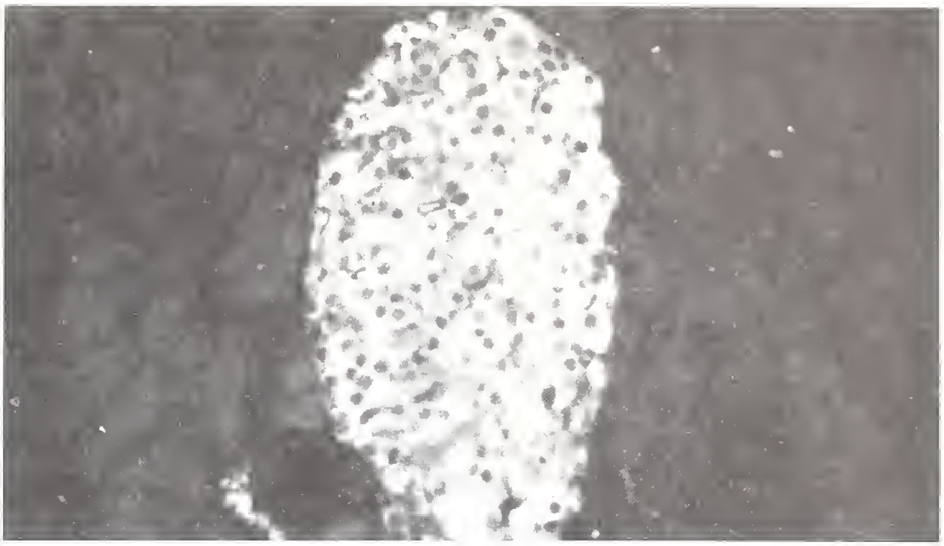
A team of scientists from the National Institute of Dental Research and the National Cancer Institute has shown for the first time that a virus can cause a disorder in several hormone-producing glands (polyendocrine disease) and induce infected animals to make antibodies to some of their own tissue, a phenomenon known as autoimmunity.

Dr. Takashi Onodera and the NIDR scientists were working with a virus known as reovirus, an acronym standing for respiratory, enteric, orphan virus. It was so named in 1959 by Dr. Albert Sabin because it was found in the respiratory tract and gut of children, but because it did not seem to produce any serious disease, it was referred to as an "orphan" virus. However, when injected into mice, it produced a number of clinical symptoms.

The NIDR team now has shown that in mice, reovirus type 1 can produce a mild type of diabetes mellitus and retardation of growth. By using a fluorescein-labeled antibody that binds to the virus, they found viral antigens in the insulin-producing beta cells in the pancreas. Also with an electron microscope they were able to identify large numbers of reovirus particles. These findings suggested that the virus, by damaging the insulin-producing beta cells, was responsible at least in part, for the diabetes.

During the course of their studies, the NIDR group also found that a high percentage of the infected animals grew very slowly. The stunted growth suggested that the virus also might be damaging the pituitary gland which produces a variety of growth-promoting hormones. Aided by electron microscopy and immunofluorescent stains, they found that cells in the anterior lobe of the pituitary, which produce growth hormone, were infected with the virus. Moreover, when they measured the level of growth hormone in the blood of the infected animals, they found it was lower than normal. They reasoned that the destruction of pituitary cells by the virus infection might account for the decrease in growth hormone and, possibly, the retarded growth.

Increasing evidence today suggests that a number of diseases, especially polyendocrine diseases, may have an autoimmune component. Most of the time, an individual's immune system is beneficial and fights off foreign invaders such as viruses, bacteria, and tumors, but under certain circumstances, the immune system may turn against and damage the individual's own tissue, a phenomenon known as autoimmunity. Last year, NIDR investigators reported that some patients with juvenile-onset diabetes have in their blood antoantibodies that react with and can destroy cultured beta cells. Similarly, patients with polyendocrine disease have autoantibodies in their blood that react with other hormone-producing cells (e.g., thyroid, gastric mucosal, adrenal, pituitary).



Section of a normal mouse pancreas incubated with serum from a mouse infected with reovirus. The photomicrograph shows brilliant staining of the autoantibodies induced by the virus in the insulin-producing beta cells.

NIH PHOTO 1745

What triggers the production of these autoantibodies is not known, but one possibility is a virus. The demonstration that reovirus could induce a polyendocrine disease in mice suggested to the NIDR team a way to develop a useful model for looking for the presence of autoantibodies. They prepared slices of pancreas, pituitary, and stomach from normal uninfected animals and then incubated these tissue sections with sera from the reovirus-infected mice. With fluorescent techniques, they found autoantibodies in the sera of reo-infected mice, but not normal mice. The antibodies reacted with beta cells, pituitary cells, and gastric mucosal cells. Other autoantibodies also were found. Detailed studies showed that one of these autoantibodies was directed specifically against insulin and another against growth hormone. Insight into the molecular basis by which reoviruses induce autoantibodies came from the fortuitous observation that another closely related virus, reovirus type 3, did not infect cells in the anterior pituitary or induce autoantibodies to growth hormone.

Reoviruses have 10 genes, and for some of these the functions are known. For example, the gene called S1 codes for a protein on the surface of the virus known as sigma 1. This protein controls the capacity of the virus to bind to and invade certain tissues. It is possible to move genes from one virus to another, and several years ago investigators at Harvard prepared a series of recombinant viruses. One of these viruses contained nine genes from reovirus type 1 and one gene (S1) from reovirus type 3. When inoculated into mice, this recombinant virus failed to infect the anterior pituitary or induce autoantibodies to growth hormone. In contrast, another recombinant virus, containing nine genes from reovirus type 3 and one gene (S1) from reovirus type 1, infected cells in the anterior pituitary and induced autoantibodies to growth hormone. From studies of these and other recombinant viruses, the NIDR team concluded that the S1 gene from reovirus type 1 is required for infection of the anterior pituitary and the induction of autoantibodies to growth hormone.

Precisely how reovirus infection leads to the development of autoantibodies is still not absolutely clear. One possibility is that the virus infection leads to the release of "hidden" antigens which the host's immune system ordinarily does not "see." A second possibility is that the virus acts on a specific subset of lymphoid cells which control the production of autoantibodies. But regardless of how these autoantibodies are induced, these and other studies in progress indicate that the individual's autoimmune response contributes to the development of disease. The demonstration that reovirus can both damage hormone-producing cells and induce an autoimmune response lends credence to the hypothesis that diabetes mellitus and other diseases with an autoimmune component may sometimes be triggered by viral infections.

This research was published in *The Journal of Experimental Medicine* 153: 1457-1473, 1981, by Takashi Onodera, Antonio Toniolo, Usha Ray, A. Bennett Jenson and Abner L. Notkins of NIDR and Richard A. Knazek of NCI. ■

Scientists Making Great Strides in Research Using Hybridomas

Bobbi Bennett

Hybridomas—the cellular factories that spew out extraordinary quantities of exquisitely specific monoclonal antibodies—are revolutionizing basic and clinical research in a host of areas. At a recent NIH Science Writers' Seminar held here, intramural scientists from the National Cancer Institute (NCI) and the National Institute of Allergy and Infectious Diseases (NIAID) described to reporters and other attendees the state of the art of this new technology in their fields.

Dr. Jay Berzofsky, a senior investigator in NCI's Metabolism Branch and moderator of the seminar, explained how hybridomas combine the specificity of the immune spleen cell with the immortality and growth capacity of the malignant tumor. Hybridomas were first produced by two scientists in England—Kohler and Milstein—in 1975 when they fused antibody-producing lymphocytes from the spleens of immunized mice with cells of a malignant myeloma tumor to produce clones of homogeneous malignant cells, all producing the same desired antibody which one could grow in virtually endless supply.

Before the advent of hybridoma technology, nothing was known about the detailed structure of the combining sites of antibodies nor of the structure of antigenic sites of proteins. The monoclonal antibodies provide a new purified chemical reagent with which to investigate these questions.

Using monoclonal antibodies, Dr. Berzofsky is studying the antigenic sites on myoglobin, an oxygen-binding protein found in muscle. He has identified binding sites in which amino acids not close together on the backbone are brought together by the folding of the protein in its native three-dimensional shape. Using 3—D projection of computer-generated myoglobin models, these "typographic antigenic determinants" could be seen, including one with a concave area in the protein's surface into which a convex portion of the antibody could conceivably fit.

On November 18, science writers from the Washington and New York areas attended an NIH-sponsored seminar on Hybridomas and Monoclonal Antibodies, Technology for the 1980's.

NIH PHOTO 1746

Dr. Jay Berzofsky, Metabolism Branch, NCI, introduced the topic of hybridomas explaining how hybridomas combine the specificity of the immune spleen cell with the immortality of a malignant cell. He emphasized the advantages of producing antibodies from hybridomas rather than from the traditional method of immunizing an animal with an antigen.

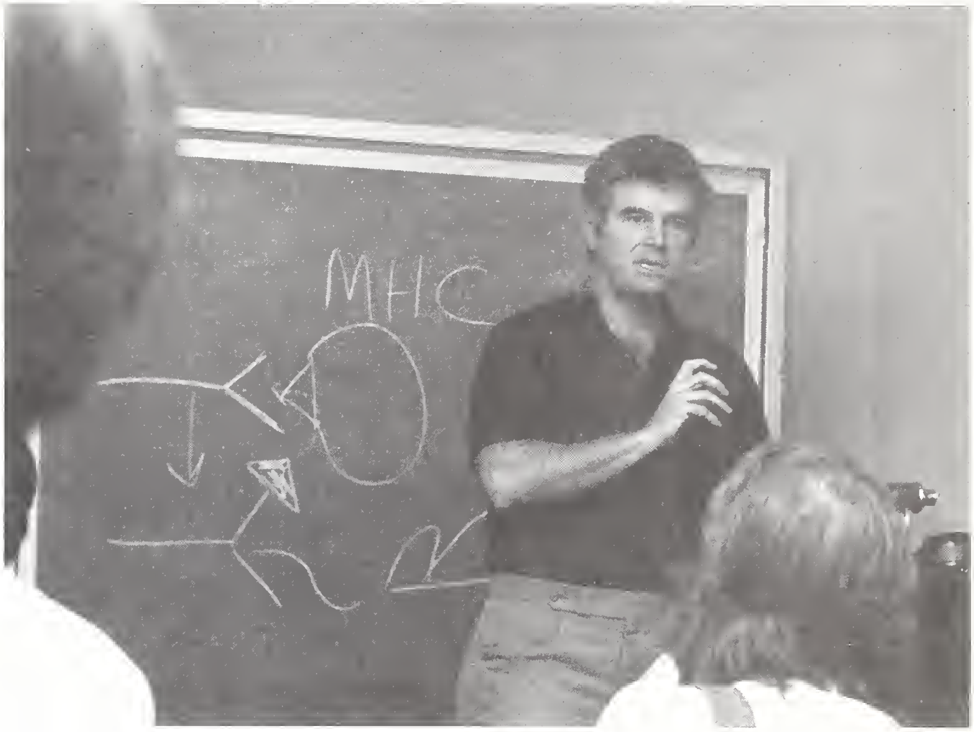
NIH PHOTO 1747

Dr. Michael Potter, chief, Immunochemistry Section, Laboratory of Cell Biology, NCI, is using this powerful research tool to see where specific antigenic determinants are on the surface of a family of mouse antibodies that all bind the same antigen (the galactan-binding myeloma proteins). By projecting 3-D images of these proteins, Dr. Potter demonstrated how an anti-idiotypic antibody—one that recognizes the antigenically unique features on another antibody—can distinguish between two galactan-binding monoclonal antibodies on the basis of a single amino acid change. The anti-idiotypic antibody was shown also to have an affinity for the environment around the antigenic determinant.

One possible clinical use of monoclonal antibodies is to specifically suppress an individual's immune response to foreign antigens on a transplanted organ while leaving the rest of the immune system intact to protect the body. Dr. David H. Sachs, chief, Transplantation Biology Section, Immunology Branch, NCI, described his studies in this field. He has found that if you immunize another species of animal with a monoclonal antibody that recognizes mouse histocompatibility antigens, the animal will make an antibody (an anti-idiotypic antibody) with a binding site that mimics, in some way, the original antigen. When this anti-idiotypic antibody is then injected into a mouse, it produces antibody as though it had seen the original antigen. These studies suggest that it may one day be possible to use anti-idiotypic reagents to specifically manipulate the immune response and block responses against transplantation antigens.

Other potential diagnostic and therapeutic uses for monoclonal antibodies were discussed by Dr. Anthony Fauci, chief of NIAID's Laboratory of Immunoregulation. Using commercially available monoclonal antibodies that recognize different functional subsets of T lymphocytes (the thymus-derived cells that mediate cellular immune reactions), scientists have been able to identify types of cellular defects seen in various diseases. This should be of great diagnostic value. Dr. Fauci has produced a heterohybridoma—by fusing human B cells (bone-marrow derived cells that make antibodies) with mouse myeloma—that is producing monoclonal human antibody without any contaminating mouse proteins. This proves the feasibility of producing antibodies to any antigen with which one could immunize a human, and then using them either to protect someone who lacks that antibody or to eliminate a particular cellular component or tumor.

Dr. John D. Minna, chief, NCI-Navy Oncology Branch, NCI, delineated the potential clinical applications of monoclonal antibodies in the diagnosis, staging, and treatment of cancer. His laboratory has developed monoclonal antibodies that can distinguish between the four major types of human lung cancer. Since 80 percent of patients die within the first year of this disease, and since there are major treatment differences between each type of lung cancer, these reagents should be of great use in the early selection of appropriate therapy. Dr. Minna has also found a heterogeneity in antigenic expression in tumors; in some, 99 percent of the tumor cells express the antigen while in others, only 10 percent may. This indicates the need to use specifically "mixed" cocktails of monoclonal antibodies to react with every bit of a tumor. His group is also working on ways to remove tumor cells from patients and screen them for sensitivity to chemotherapeutic drugs as well as to monoclonal antibodies. ■



Dr. David Sachs, Chief, Transplantation Biology Section, NCI, explained to the science writers that an animal immunized with a monoclonal antibody that recognizes mouse histocompatibility antigens will produce an antibody with a binding site that mimics the original antigen.

NIH PHOTO 1748

Consensus Members Agree that CT Scanning is a Powerful Diagnostic Tool—Safe and Efficient

Diane Striar

Computed tomographic (CT) scanning of the brain is a safe, accurate, and powerful tool in the primary diagnosis of brain tumors, brain hemorrhage, major head injury, and certain brain infections, agreed members of a blue-ribbon panel at a recent NIH Consensus Development Conference on "CT Scanning of the Brain." CT is a remarkable imaging technique that combines x-ray equipment with a computer and television-type display.

However, the Consensus Development Panel cautioned in its draft report, that CT should not be employed as a "routine screening procedure" when patients show little likelihood of structural disease such as in minor head trauma or simple headache.

These recommendations followed an intensive two-day conference sponsored by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the National Cancer Institute.

"The time was ripe for a Consensus Development Conference," remarked conference organizer Dr. Michael D. Walker, NINCDS Stroke and Trauma Program director. "The field has stabilized, and enough data has accumulated in the last 10 years, to allow an in-depth exploration of the CT."

The 13 scientists and health administrators charged with assessing the scanners concluded that the machines have been a major factor in decreasing deaths—especially in severe head injury and brain abscess. The presence of these conditions clearly calls for the diagnostic assistance of the CT, according to the panel.

Other indications for CT use specified by the panel include suspicion of arteriovenous malformations, hydrocephalus, herpes simplex encephalitis, parasitic infestations, progressive degenerative diseases of the brain, and intracranial tumors. In primary brain tumors, the use of CT has resulted in the detection of smaller lesions, lower death rates following surgery, and decreased time in the hospital.

The panel also concluded that CT will usually differentiate between ischemic and hemorrhagic intracranial lesions—helping the physician to select appropriate medical or surgical therapy.

Another use for CT is the identification of a potential structural cause of complex partial or focal epileptic seizures.

The panel found CT to be an important clinical tool in pediatric neurology that should be used to evaluate undiagnosed coma and a number of other neurological symptoms.

Although panel members acknowledged that CT carries potential hazards, including a small risk of adverse reactions to contrast material if used, their report concluded that the procedure is "remarkably safe." The amount of radiation produced by the CT is comparable to or less than that emitted from many other routine diagnostic procedures, including dental x-rays, the panel reported. A complete scan emits 1 to 10 centigrays of radiation to the brain. The "gray" is the new unit by which radiation dose is expressed, replacing the "rad" (1 gray = 100 rads; 1 centigray = 1 rad).

The panel also suggested that physicians use special caution in ordering multiple scans for children since "the effects of repeated cumulative low-level radiation doses to the immature, developing brain are as yet unknown."

Charged with assessing the impact on other techniques for "imaging the brain," the panel found that the CT scan's superior ability to detect intracranial and intracerebral disease has led to the decreased use of other radiographic methods such as skull roentgenography and cerebral angiography.

In line with the panel report, panel chairman Dr. Fred Plum, who is chairman of the Cornell University Medical College Department of Neurology, asserted that

EMI Brain Scanner. The patient is placed on a table with her head in the opening. A pencil-thin X-ray beam passes through the head and is picked up on the opposite side. The computer calculates the attenuation of the beam, giving density readings at 28,000 points for each "slice", then projects a picture of each slice onto the screen. A circular apparatus moves forward slightly and performs another scan. Blood clots, hemorrhages and other cerebrovascular disorders can thus be detected. (NIH, NINCDS Computed Tomography Section-July 1971).

NIH PHOTO 1749

"any cost imposed by the use of CTs has been offset by the reduced costs resulting from the declining use of other scans." Furthermore, according to evidence presented to the panel, CT use in outpatient diagnosis has reduced hospital admissions and shortened hospital stays.

CT costs may be reduced even further if physicians heed the panel's advice concerning the criteria for machine use.

"CT is not the neurologist's chest film," said Dr. Plum. "The application of this new technology is probably most overused in headache diagnosis," he added.

According to the panel report, only patients who have severe, constant or unusual headaches or those associated with abnormal neurological signs should be considered for CT scanning.

The panel also voiced concerns about the availability of CT scans. Although there are 1,400 scanners in the U.S. today, the scientists concluded that CT "may not be sufficiently available for the public to derive the full benefit of its potential."

The panel felt there might be an insufficient number of instruments in some large metropolitan areas, in medically underserved areas, and in sparsely populated regions which experience a high incidence of head trauma. The list of hospitals without scanners includes Harlem Hospital and Metropolitan Hospital in New York City and Cook County Hospital in Chicago.

The inadequate number of CT scanners in the U.S. may be due not only to the cost of the equipment—from $150,000 to over $1 million—but also to the elaborate regulatory process required of hospitals and physicians who wish to buy them. The "certificate of need" that is required for the purchase of scanners is obtained through a public process that includes hearings and may cost well over $100,000, according to Dr. Plum.

"One hospital in Connecticut paid 20 percent of the costs of the machine just to get permission to buy it," added Dr. Hillier L. Baker, panel member and professor of radiology at the Mayo Clinic.

"Both state and Federal regulatory processes have placed enormous restraints on hospitals getting scanners," said Dr. Plum. "These restrictions pose the greatest obstacle to the growth of the use of scans in the U.S." ■

Initial Testing of Meningitis Vaccine Shows Promise

Prepared by Baylor College of Medicine, Office of Public Affairs

Researchers at Baylor College of Medicine in Houston have completed initial testing in adults of one of the first vaccines which shows promise in preventing group B meningococcal disease.

Meningococcal disease is one of the more common causes of meningitis in young children, particularly under the age of two.

Meningococci are classified in groups according to the polysaccharide content of the organism's capsular wall. Groups A, B, and C cause the majority of illnesses accompanying infection with this organism. Vaccines containing the polysaccharides of groups A and C have already been developed and licensed for use in preventing infection. Both vaccines elicit protective responses (antibodies) in children as well as adults. However, efforts to produce a similar vaccine for the group B meningococcal organism have been unsuccessful to date.

In a paper presented to the American Society of Microbiology on November 6, John M. Zahradnik, M.D., Assistant Professor of Microbiology and Immunology at Baylor College of Medicine, reported meningococci also have an outer membrane protein which can stimulate antibody production, in addition to this group specific polysaccharide. The serotype 2 protein was selected for investigation as it is found in many meningococci causing disease.

The Baylor study—supported by the National Institute of Allergy and Infectious Diseases—evaluated two meningococcal B preparations developed by C.E. Frasch, Ph.D., of the Bureau of Biologics, Bethesda, Maryland. One contained only the serotype 2 protein, while the other was a combination of the serotype 2 protein and the group B capsular polysaccharide.

The test group included 50 adults, age 20-37 years, who received two intramuscular doses of the vaccine four weeks apart. Volunteers were seen twice during the 48-hours following each injection. Blood specimens were taken throughout the six weeks of the study.

Zahradnik reported most of the recipients of the vaccine exhibited significant antibody response. The combined protein-polysaccharide vaccine resulted in higher antibody levels and a greater frequency of antibody response than the protein vaccine alone.

Most of the volunteers experienced mild-to-moderate local pain. This side effect lasted only about 24 hours and was much milder following the booster dose. Other patients experienced systemic reactions, especially after receiving the protein-polysaccharide vaccine.

Zahradnik concludes that it appears this meningococcal group B vaccine stimulates significant antibody production in adults, particularly when the serotype 2 protein is combined with the group B meningococcal polysaccharide.

Also participating in the study was Thomas R. Cate, M.D., Baylor College of Medicine. ■

Radiation Therapy Brings Relief to Arthritis Sufferers

Connie Raab

The crippling symptoms of severe rheumatoid arthritis can be significantly reduced in patients treated with a form of radiation therapy developed for Hodgkin's disease, according to two preliminary studies.

The technique, known as total lymphoid irradiation or TLI, involves irradiation of the lymphoid tissues, including lymph nodes, the thymus, and the spleen. Researchers in California at the Stanford University Medical Center and in Boston at Harvard Medical School and Brigham and Women's Hospital used TLI to treat a total of 21 patients with severe rheumatoid arthritis which was unresponsive to conventional treatment. All had highly active arthritis and most were too disabled to care for themselves or work. With the irradiation therapy, 17 of the patients had a marked reduction in joint inflammation, along with a reduction in joint pain and increase in function.

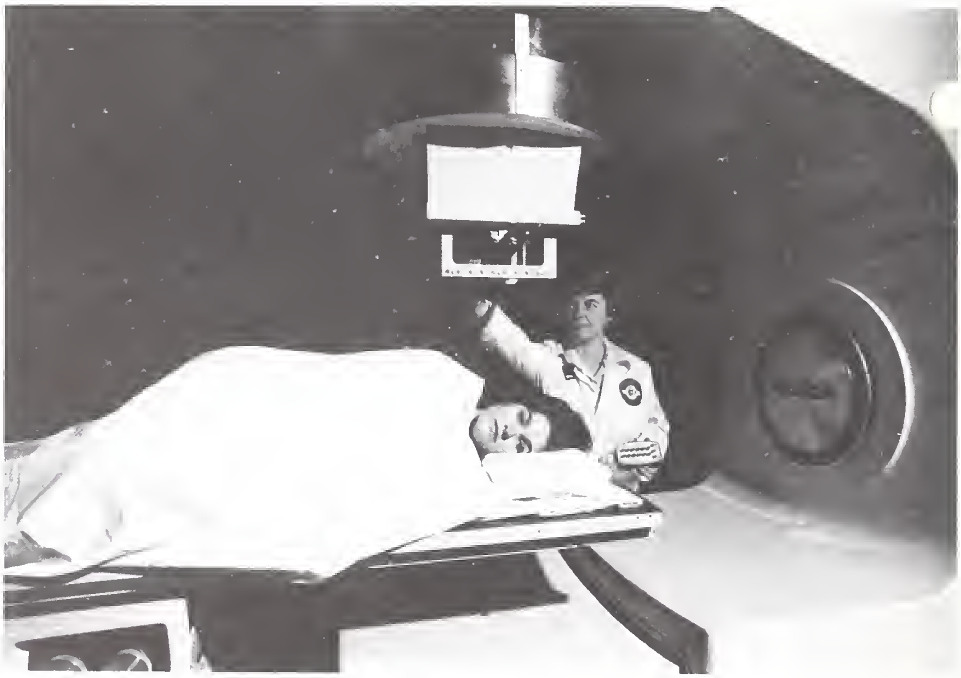
For the past two decades, TLI has been a safe and effective treatment for Hodgkin's disease, a form of cancer. In 3,000 Hodgkin's patients followed ten years after treatment there were no serious, long-term side effects of the treatment and the development of secondary cancers (including leukemia) was extremely rare.

TLI is known to suppress the immune system, normally the body's defense against foreign substances. In rheumatoid arthritis, and other so-called autoimmune diseases, the body's "defenses" seem to attack its own tissues leading to joint inflammation and tissue destruction. In an interview with the Arthritis Foundation, Dr. David Trentham, head of the Boston group explained why they used TLI: "The low toxicity of the radiotherapy technique, which has been well refined over the past 15 years, make this a far less dangerous way to suppress the immune system than cytotoxic agents," powerful drugs that are known to have serious side effects.

The Stanford researchers, led by Dr. Samuel Strober, treated 11 patients with a total of 2,000 rads given first to one half of the lymphoid area in small doses over a two to three week period, immediately followed by a series of small doses over a second two to three week period. Nine of the 11 patients showed a significant reduction in joint tenderness and swelling, generally beginning one month after therapy. Improvement peaked at six to seven months and remained at that level throughout the followup period (for one patient, 18 months). Three of the patients had temporary flareups and were treated by drugs, but none of the nine patients showed any long-term tendency for the disease symptoms to return.

The results of the Boston study, which used the technique somewhat differently, were also impressive; the suppression of arthritis, however, lasted for only several months. The Boston researchers treated ten patients with a series of small doses totalling 3,000 rads, treating one-third of the lymphoid field over three to three and a half weeks with a two week rest period before the next third of the lymphoid area was treated. The entire course of therapy took 13 to 15 weeks. The investigators found significant improvement in eight of the ten patients, with the reduction in symptoms often beginning during the latter part of therapy. In six of the eight patients followed for 17 to 21 months, joint swelling returned within a year, although five of the six remained better off than before starting therapy.

In both studies, investigators found that improvement in the symptoms of rheumatoid arthritis was accompanied by a reduction in reactivity of T-lymphocytes, a kind of white blood cell involved in the regulation of immune responses. Of interest was the fact that there were no reductions in blood levels of autoantibodies, such as rheumatoid factors and antinuclear antibodies, or of circulating immune



An arthritis patient is being set up to receive radiation treatment from a high-energy X-ray machine.

NIH PHOTO 1750

complexes (combinations of antigens and antibodies.)

In an editorial accompanying the reports, Dr. Daniel McCarty of the Medical College of Wisconsin, said that "technical differences in [the administration of] total lymphoid irradiation could explain the difference in results" between the two studies. He estimated that about 100,000 of the approximately six million adults with rheumatoid arthritis in the United States have disease uncontrolled by conventional treatment which could lead to severe impairment. TLI is one of a few new treatments (including cytotoxic drugs) that might be effective for these patients; all of these therapies are still experimental. Dr. McCarty urged the news media to "responsibly report that total lymphoid irradiation, while promising, needs controlled evaluation by a few investigative groups using carefully designed protocols before it is generally used in intractible rheumatoid arthritis."

The investigators also stress that it is premature to recommend TLI as a form of treatment, even for severe rheumatoid arthritis, before further studies are done. However, they feel the research sheds light on the role of T-cells in immunoinflammation, and may lead to treatments that more specifically suppress T-cell function, rather than the immune system as a whole.

The Stanford study was supported by grants from the National Institute of Allergy and Infectious Diseases (NIAID) and the National Cancer Institute (NCI). The Boston study was supported by grants from the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (NIADDK) and the Division of Research Resources. Their findings were reported in the October 22, 1981, issue of *The New England Journal of Medicine.* ■

New Report Provides Cancer Rates by Region

Linda Anderson

A new report from the National Cancer Institute provides comprehensive data on annual cancer rates for 1973-1977 in the United States. The information is based on newly diagnosed cases of cancer and deaths, collected yearly for five states, five metropolitan areas, and Puerto Rico by the NCI's Surveillance Epidemiology and End-Results (SEER) program.

The report—*Incidence and Mortality Data: 1973-1977*—provides the most extensive profile available on the ocurrence of new cancer cases and deaths, according to body site and histology (cell type), and by sex, age, race, and geographic area of the groups affected. It presents the first detailed data available on the occurrence of cancer among certain groups of American Indians, Hispanics, Hawaiians, Filipinos, Chinese, and Japanese, in addition to information on blacks and whites.

Variations in the occurrence of cancer among the population have been noted by scientists who developed and use SEER data. This report provides others with access to data that may be used in designing studies to examine possible reasons for these differences. The following 11 geographic areas are covered by SEER: Connecticut, Iowa, New Mexico, Utah, Hawaii, Atlanta, Georgia, New Orleans, Louisiana, Detroit, Michigan, San Francisco, Oakland, California, Seattle-Puget Sound, Washington, and Puerto Rico.

The report presents the data in a variety of ways and at different levels of detail. Data on newly diagnosed cancer cases were averaged for the five-year period, and expressed as average annual rates of the cancer incidence per 100,000 population. Data on deaths due to cancer were averaged and expressed as mortality rates.

Cancer incidence and mortality rates for the combined areas, excluding Puerto Rico, are reported for whites and blacks. Although the black population was not sufficiently large to determine rates separately for each geographic area, the total black population was large enough to report rates for the combined areas, excluding Puerto Rico.

The incidence rates presented for the combined areas, excluding Puerto Rico, may be used as indicators of the estimated rates of occurrence of cancer among the United States population. Cancer mortality rates are available for the total United States and are presented, where available, in the same tables as the mortality rates for the combined areas, excluding Puerto Rico.

Data on the histology of all cancers (microscopically confirmed) are presented only for the combined areas, excluding Puerto Rico. The histologic type is of importance because different types of cancer may occur within the same site, and prognosis often depends on the precise diagnosis.

The Surveillance Epidemiology and End-Results Program is a major source of data on the occurrence of cancer in the United States. National estimates of the current number of cases diagnosed annually are based largely on these data.

The report was prepared by the Demographic Analysis Section, Biometry Branch, Field Studies and Statistics, Division of Cancer Cause and Prevention, NCI. Single copies are available free of charge from the National Cancer Institute, Office of Cancer Communications, Building 31, Room 10A18, Bethesda, Maryland 20205. ■

Testing Drugs: Process and Pitfalls

Ray Fleming

Haloperidol. Levodopa. Baclofen. Detoxified snake venom. Valproic acid. All represent substances tested in an attempt to ease the disability and suffering caused by neurological diseases. Some have proven effective; others remain controversial. Regardless of their status, all are objects of intense interest to physicians seeking better treatments and to patients who may benefit from them.

Unless a new drug produces a dramatic, positive result in nearly all cases of a disease with a predictably poor prognosis, it must be subjected to a clinical trial. Without such a trial, investigators may have difficulty determining which changes, if any, in a patient's condition can be attributed to the drug, and which would occur naturally in the course of the disease.

Scientifically establishing the therapeutic effects of drugs on the human body is much more complicated and difficult than most people realize. The length and cost of drug trials can seem excessive to patients awaiting the results, and poorly designed therapeutic studies may lead to disappointing outcomes. Understanding the process of drug testing and the problems to be solved along the way can help patients and their families put their concerns in perspective.

Dr. Bruce Schoenberg, chief of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) Neuroepidemiology Section and an expert in the design and evaluation of drug trials, believes that the success of almost any test depends upon choosing the correct variables to measure. When studying a new drug for use against amyotrophic lateral sclerosis (ALS), he points out, it will do little good to measure the drug's effect on leg muscle strength if patients are deteriorating because of swallowing difficulties. The choice of measurement, in other words, must be relevant to the continuing course of the disease.

In choosing a particular measurement, the scientist must also consider how accurate and reproducible it is. How closely can a measurement gauge a particular characteristic? Can the results be repeated by others? Or even by the same scientist? If not, the results can easily be challenged, and months of work may be wasted.

Dr. Schoenberg warns that biased measurements and observations, whether they involve the patient, the scientist, or both, must also be avoided. "In some situations, patients must evaluate themselves, and they may subjectively feel better or report improvement if they know they are receiving a new form of therapy," he explains. "Even the investigator may be influenced in his or her measurements by the knowledge that a patient is receiving a particular drug. In hope of finding an effective treatment, the scientist may give patients receiving the drug a more optimistic evaluation."

To reduce bias in a study, scientists rely on three types of tests:

- Single-blinded study. The investigators know which patients are receiving the drug being tested, but the patients do not. In some patients a placebo may be substituted for the drug under study.
- Double-blinded study. Neither the person measuring the response nor the subjects know which patients are receiving the new drug and which have been given placebos. That information is coded.
- Triple-blinded study. The identity of treated individuals is kept from the subjects, the person measuring the response, and the data analyst. Coded identities are revealed only after the data have been evaluated.

When judging the effectiveness of a particular drug, the scientist usually makes comparisons to arrive at a conclusion. Does drug A work better than treatment B? Than no treatment at all? To answer these questions, most drug investigators also monitor a group of "control" subjects during the trial—patients who are given a placebo or another treatment in place of the drug being tested.

Much care should be taken, says Dr. Schoenberg, in selecting subjects for a drug trial and assigning them to control or new drug groups. To prevent known causes of bias from influencing the outcome of the tests, subjects should be as similar as possible in age, sex, length and severity of illness, and other characteristics. Finding suitable patients can be a difficult and time-consuming process, but it is a necessary one. Once similar subjects are chosen, the scientist attempts to remove unknown sources of bias by randomly assigning individuals to study or control groups.

The drug trial itself can be designed in several different ways. In the "concurrent parallel" test design, a subject assigned to either the control or study group remains in that group throughout the investigation; both groups are monitored over the same period of time.

Another popular design sometimes used is the cross-over study, in which patients serve as their own controls. After participating in a trial as either study or control subjects, patients are taken off the medication or placebo for a time to allow the body to eliminate the effects of treatment. The two groups are then switched; those who had received the test drug now receive the control treatment or placebo, and vice-versa. Cross-over studies generally require fewer patients, are less expensive, and have the additional advantage eventually offering the new treatmen all test subjects.

Regardless of how carefully measurements and subjects are chosen and used in a drug trial, delays and difficulties may still arise. Dr. Schoenberg mentions the difficulties caused by test volunteers who may refuse to participate in the trial at the last minute, those who withdraw after the test has begun, and those who develop, other medical problems during the course of the trial. Some test subjects may not be available for important follow-up studies.

Drug trials carried out at more than one location pose additional problems. Measurement and recording techniques must be standardized, and systems devised to verify that patients are receiving medication according to plan. This can be particularly difficult in a home situation when the subject or the subject's family is responsible for giving the drug.

Data analysis can be the most error-prone area of all. At what point, for example, should the scientist evaluate the results? If statistical tests are applied to the data whenever the results look interesting, the drug can appear to be more effective than it actually is. Is the sample size large enough? Generally, the smaller the drug effect to be measured, the larger the sample size required. Some advocat of speedier drug trials have suggest combining the data from all published results about the same drug. But even if the various studies were compatible, the combined data would present an overoptimistic view: drug trials with poor results do not always find their way into print.

It is also important to remember that the results of even the most perfectly designed and executed drug evaluations can only be applied to the types of patients studied in the test. A successful trial does not necessarily mean that a new compound will be effective for all patients.

"That's why it's essential for investigators to state precisely their diagnostic criteria and rules for the inclusion and exclusion of study subjects," says Dr. Schoenberg. "This will permit others to replicate the trial or decide whether the results are applicable to their own patients."

Few events in science generate as much excitement and anticipation as the discovery of a new drug, and in the next decade we can expect the revolution in genetic engineering to produce even more compounds with the potential for treating neurological diseases. With careful investigator planning and strong s port by educated health-care consumers, the drug trial will continue to be a touchstone of drug effectiveness on which we can rely. ■

Hormones Play an Important Part in Growth

Marian Segal

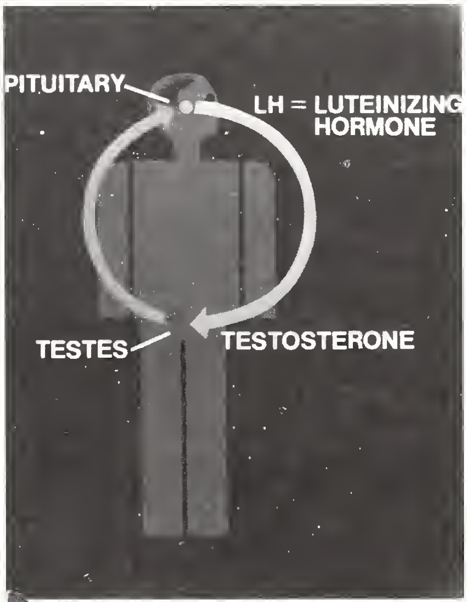
Children grow at different rates. This is normal and to be expected, and the differences are never more apparent than during puberty.

Some adolescents seem to tower over their classmates while others take the front row for class pictures so they won't be lost behind their peers. Although the differences are most exaggerated during these years when development occurs in rapid growth spurts, growth rates vary throughout childhood.

These variations are normal for the most part, but occasionally growth may be impeded or accelerated beyond the established range of normal. What is too tall? What is too short? What should parents and pediatricians be alert to in assessing children's growth?

These were the questions Dr. Barry Bercu addressed in his "Medicine for the Layman" talk November 10. Dr. Bercu is the head of the Pediatric Endocrine Unit, Neonatal and Pediatric Medicine Branch, NICHD.

He explained how the endocrine system regulates growth and discussed how parents and pediatricians can be aware of possible developmental problems. The endocrine glands, such as the pituitary, thyroid, adrenals and gonads (ovaries and testes) produce hormones that directly or indirectly affect height, weight, and sexual maturation.



An endocrine "feedback system" works to reduce hormone production and secretion when the substance reaches a certain level in the blood and increases it again when the level falls. NIH PHOTO 1751

These hormones are secreted into the bloodstream and circulate to their "target organs" where they regulate cellular activities involved in growth. Operating on the same principle as a thermostat in raising or lowering room temperature, an endocrine "feedback system" functions to reduce hormone production and secretion when the substance reaches a certain level in the blood and increases it again when the level falls.

To illustrate the role of hormones in growth, Dr. Bercu focused on the action of human growth hormone (hGH), which is produced by the pituitary gland. A part of the brain called the hypothalamus, which sits directly above the pituitary, produces a chemical substance that signals the pituitary to produce or release hGH.

The secreted hormone travels to the liver, its target organ, and induces production of another hormone called somatomedin.

Somatomedin in turn acts on the growth plate centers at the ends of long bones to stimulate bone growth. With this, the bone lengthens and height increases.

Problems can arise at various points in the system, however. For example, overproduction of hGH due to a pituitary tumor can cause too much growth, resulting in gigantism. Conversely, if the hormone is produced in insufficient quantity or not at all, dwarfism results.

Other problems, such as malnutrition, major organ disease or other chronic illness, emotional deprivation, or chemical defects at the cellular level can also impede normal growth.

Based on studies of large numbers of children, scientists have compiled data on normal growth patterns. Expressed in growth charts, the data can be used by pediatricians to trace children's growth progress and determine if they are within the normal, or expected range of height and weight at a particular age.

For the 2 percent of children who do not measure within this range, the pediatrician and pediatric endocrinologist try to determine whether the child's development is normal or abnormal.

Growth is seasonal and children grow more rapidly from spring to summer than in fall and winter. Thus, at least 1 full year of measurements is needed to collect reliable data. Growth is influenced by genetics as well. Tall parents tend to have taller children, and the reverse is true for short parents.

Research is under way to treat many growth abnormalities. Perhaps the most exciting recent development is the use of recombinant DNA technology to produce human growth hormone which, until recently, was available only from cadavers and thus in extremely short supply.

Production of this hormone in large amounts will bring down the cost of treatment considerably and open the possibility of therapy for many children who might benefit from it. ■

Midget wrestler, Lord Littlebrook, was a champion in his field during the 1960's despite his limited growth.
NIH PHOTO 1752

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News&Features from NIH

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

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News&Features from NIH

February 1982

NIH Clinical Center physicians have developed new methods of blood transfusions that may be safer and more practical in treating patients with sickle cell anemia. Removing a patient's blood and simultaneously replacing it with normal blood is a more effective way of raising the percentage of normal red blood cells than is conventional transfusion. Unlike conventional transfusion, exchange does not overburden the body by repeatedly adding blood cells to those already present in the recipient's own system. See page **2**.

The drama and sophistication of coronary bypass surgery is captured in a series of photographs taken at the NIH. More than 100,000 of these operations are performed annually, to improve life expectancy and the quality of life for victims of coronary heart disease. Cover story page 8.

Location of body fat in women may be a reliable indicator of undiagnosed diabetes according to a recent NIH-supported study. Women with upper body fat have a significantly greater chance of developing diabetes than their counterparts with excess lower body weight. The excess weight above the waist seems to be caused by enlarged fat cells that fail to recognize insulin, resulting in an inability to use glucose effectively. See page **4**.

Medical treatment of noncancerous disease with radiation was more prescribed 40 and 50 years ago, before the dangers of radiation were known. An NCI study of radiation reveals some interesting facts about cancer risk and the development of cancer in general. For instance, other than dose, age at exposure may be the most important determinant of future cancer risk. See page **10**.

An excess number of cases of malignant melanoma was found in employees at a nuclear research laboratory in San Francisco. Although lab workers appear to be the occupation most at risk, the researchers have no conclusive evidence that work environment is the cause. See page **7**.

Studies of spinal cord injury in hibernating squirrels are revealing valuable clues about how damaged nerve cells in the central nervous system can regenerate. Grantees of the Neurology Institute believe that their work will offer hope to paraplegics and other victims of spinal cord injury. See page **6**.

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Editorial Staff

Mary Donovan
Editor

Susan Gerhold
Associate Editor

Helen Ou
Special Assistant

Maureen Napper
Editorial Assistant

NIH News Branch
Building 31, Room 2B-10
Bethesda, Maryland 20205
Phone:
(301) 496-2535

Exchange Transfusion Offers Hope for Sickle Cell Patients

Charlotte Armstrong

Scientists in NIH's Clinical Center Blood Bank and the National Heart, Lung, and Blood Institute are developing and testing new methods of blood transfusion for sickle cell anemia patients which promise to make the procedure more practical and safe for treating the complications of this disease.

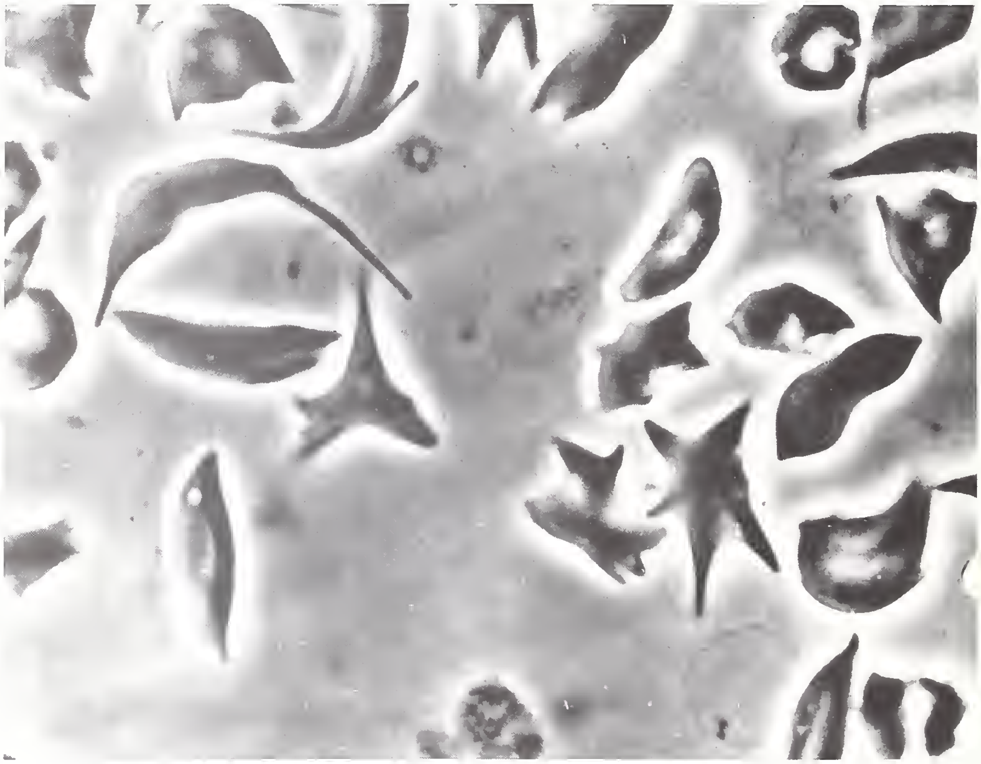
Research studies conducted by Dr. Harvey Klein in the Clinical Center Blood Bank and colleagues in the National Heart, Lung, and Blood Institute are focusing on exchange transfusion in which a patient's blood is removed and simultaneously replaced with normal donor blood. This transfusion method is more effective in raising the percentage of normal red cells in blood than in conventional transfusion.

An estimated 50,000 Americans, most of them black, have inherited an abnormal form of hemoglobin, known as sickle cell anemia, due to the distorted shape of the red blood cells. Sickled cells cannot pass easily through small vessels and they may hinder or block circulation, damaging tissues all over the body. Sickled cells are also more fragile than normal red cells and become damaged in the course of circulation. The resulting anemia may be severe.

Although the concept of exchange transfusion is not new, only recently have machines made it possible to do such transfusions over a practical time period, on the order of hours instead of days.

Dr. Klein, Dr. Robert Winslow (NHLBI), and several other investigators were the first to use an exercise system and physiological measurements to demonstrate that oxygen delivery to tissues was increased in patients with sickle cell disease who had undergone exchange transfusion. Previous reports of improvement after exchange transfusions had been based on the clinical impressions of physicians rather than measures of physiological function.

Exercise performance provided the NIH group with an easy to measure indicator of oxygen delivery to skeletal tissues. With exchange transfusion, patients' blood was maintained with 50 percent normal hemoglobin and 50 percent sickle cell hemoglobin. The overall fraction of hemoglobin in the blood, however, was kept constant so that any change in the patients' exercise performance would be due to the difference in percentage of the normal and sickle cell hemoglobin, not in the overall amount of hemoglobin in the blood.



The distortion of red blood cells in sickle cell anemia gives the disease its name.
NIH Photo 1753

Exchange transfusion increased the ability of each of fourteen patients who underwent exercise testing to perform physical exercise on a stationary bicycle. The increase was measured by comparing the amount of work the subjects were able to perform before they reached a point—the anaerobic threshold—where their body could not supply muscle tissues with enough oxygen to meet the demand. Blood tests for a side product of anaerobic metabolism, lactic acid, provided an indicator of when subjects reached the anaerobic threshold during exercise.

Dr. Klein cautions that these results cannot be interpreted as demonstrating that exchange transfusion is helpful in treating the symptoms and complications of sickle cell disease. However, the researchers reason that delivery of oxygen to tissues such as skeletal muscle and the clinical manifestations of the disease are related and that improving oxygen delivery would be helpful in controlling sickle cell complications.

Although the procedure is not free of the risks associated with conventional transfusion, it does reduce the risk of one major hazard of repeated transfusion—iron overload. This potentially lethal condition occurs when the body cannot rid itself of the iron left when red blood cells are destroyed. Unlike conventional transfusion, exchange does not overburden the body by repeatedly adding blood cells to those already present in the recipient's own system.

A blood cell separator is used to perform exchange transfusion in which a patient's red blood cells are removed and replaced with normal cells from donated blood. NIH Photo 1754

Refinements of exchange transfusion may eventually lower the risk of encountering other side effects of transfusion. In addition to continuing to observe the patients now receiving exchange tranfusion to see if the benefits outweigh the risks over the long term, the researchers are also looking for ways to reduce the frequency with which the procedure has to be performed and still be useful in controlling complications of sickle cell.

Decreasing the frequency of transfusions would lower the risk of exposure to hepatitis, a liver inflammation which can be transmitted by transfusion. Less frequent transfusions would also reduce the possibility of sensitization of a transfusion recipient to antigens on the blood cells of donor blood. Some people who have had many tranfusions have developed a sensitivity to so many different blood antigens that they can no longer undergo transfusion.

One concept which may prove beneficial for sickle cell patients is the transfusion of young red blood cells called "neocytes." Because neocytes are younger than most red blood cells, a unit of blood with a high concentration of neocytes will survive longer than a standard unit of blood when transfused. As a result, transfusions are needed less frequently.

Neocytes are collected from normal donated whole blood by centrifugation. The neocytes are lighter than normal blood cells, so that when whole blood is spun in a centrifuge, the neocytes collect near the top of the red blood cell layer.

The NIH workers have tested three different commercially available cell separator machines to arrive at an effective method for collecting neocytes from donor blood. To efficiently collect neocytes, the researchers found they required a cell separator in which the speed of the centrifuge could be controlled. They also had to develop a means of "tapping" the portion of the blood which would provide both a high percentage of neocytes and a high hematocrit or concentration of red blood cells. Tapping the blood higher and higher in the collection vessel yielded a high percentage of neocytes, but lower and lower hematocrits because the higher the tap, the more liquid plasma from the top layer was collected. They found that it was best to use hematocrit as a guide in deciding at what level to tap blood in order to collect units with adequate red blood cell content as well as neocyte concentration.

Transfused neocytes have been demonstrated to survive much longer than units with normal blood. One patient with thalassemia, another inherited and severe form of anemia, was supported using neocyte transfusion for almost six months.

Collection of neocyte units is expensive, but could substantially reduce the requirement for transfusion in sickle cell patients who require frequent transfusion therapy. The NIH investigators are continuing to seek more practical methods for collecting young cells.

Another means of reducing the frequency of exchange transfusion which the NIH researchers are looking at is selective removal of sickle blood from patients.

Ideally, exchange transfusion would replace 100 percent of a person's sickle cells with 100 percent normal blood cells. In practice, this is very difficult to do. As an exchange progresses, the patient has a higher and higher percentage of normal hemoglobin in his or her blood. Blood removed from the patient during the exchange also contains a higher and higher percentage of normal hemoglobin. Thus, as exchange continues, the process becomes more and more inefficient.

Although red blood cells with sickle hemoglobin will separate out to some extent from the rest of blood during centrifugation, it is difficult to tell the exact percentage of sickle hemoglobin collected in this manner. The NIH group is now experimenting with deliberately deoxygenating the blood *ex vivo* (outside the body) to induce sickling of cell with sickle hemoglobin. These cells could then be removed more easily from normal blood by either centrifugation or filtration. Deliberately stimulating sickling *ex vivo* is now being studied for its potential effects on other blood components such as platelets and white cells. If it proves to be useful without causing harmful effects, it could be applied in the future with patients.

New methodologies may make it possible to use transfusion to prevent as well as treat complications of sickle cell. As with other ongoing research on sickle cell, the goal of this work is to make treatment of symptoms as effective and safe as possible until a means is found to correct the inherited biochemical defect that is the underlying cause of the disease. ■

Location of Body Fat Can Predict Undiagnosed Diabetes in Women

Office of Public Relations, The Medical
College of Wisconsin

"Location of fat in overweight women is an indicator that they may be undiagnosed diabetics and a predictor of their success on any diet," according to Ahmed H. Kissebah, M.D., Ph.D., professor of medicine and director of the General Clinical Research Center at the Medical College of Wisconsin (MCW).

MCW researchers have shown that apparently healthy women in whom fat is localized in the upper half of the body have a significantly greater chance of having undiagnosed diabetes than their counterparts in whom the excess fat is located below the waist.

"What is heartening," according to Dr. Kissebah, "is that it is relatively easy to identify those women at risk and to take early measures to reduce upper body segment fat. This reduction lowers the risk of such metabolic disorders as diabetes as well as high blood pressure and heart disease."

Women with lower body segment obesity, while showing little or no risk of diabetes, are faced with greater frustrations in dieting. "Their strictest efforts result in weight loss in the upper body with virtually no change in lower body fat," Dr. Kissebah says.

The six-year study took place at MCW's General Clinical Research Center, a special human studies unit of the Division of Research Resources of the National Institutes of Health (NIH). It is located at Milwaukee County Medical Complex. The study was also supported by TOPS (*T*ake *O*ff *P*ounds *S*ensibly) Club Obesity and Metabolic Research Program.

The association of known diabetes with upper body segment obesity was first identified by French researchers in the '70s. This relationship was confirmed by MCW researchers in 1980 after studying 15,500 obese women enrolled in the TOPS program in the United States.

The current study was undertaken to predict how many otherwise healthy women have undiagnosed diabetes. This is important because the majority of diabetic persons don't manifest symptoms in the early phases of the disorder, Dr. Kissebah says. By predicting diabetes, intervention with diet, exercise and medication can begin early, delaying the ultimate ravages of the disease.

Unlike men, who mostly have their weight above the waist, women show several patterns of fat distribution. They may be lower body obese with excess weight around the hips, buttocks and thighs; upper body obese with excess weight around the waist, chest, neck and arms; or any combination of these.

To study the relationship of diabetes to upper body obesity, the researchers screened 52 women; 25 upper body obese and eighteen lower body obese. They were matched for degree of weight, height and age. Nine women of normal weight served as the control group in the study. The researchers found that the women with upper body obesity had very high blood levels of glucose, insulin and fatty acids, which usually precede the occurrence of diabetic symptoms.

To understand why this problem occurs in women with upper body obesity, the researchers examined fat and muscle biopsies taken from the abdomen of upper body obese women and from the thighs of lower body obese women. An analysis showed that the excess weight above the waist was due to the overpacking and enlargement of fat cells which do not recognize insulin, resulting in the cells inability to utilize glucose effectively.

Initial studies at the Clinical Research Center have shown that the large fat cells have a smaller number of insulin receptors, which may explain why these cells resist the action of insulin.

Dr. Ahmed Kissebah removes a fat biopsy from a volunteer normal control patient at the Medical College of Wisconsin General Clinical Research Center. The biopsy will be checked for the size of its fat cells, which has been linked to the frequency of undiagnosed diabetes, according to Dr. Kissebah. NIH Photo 1755

In contrast to the enlarged fat cells above the waist, the fat cells in the lower body obese women were shown to be normal in size; their obesity being due to increased numbers of fat cells.

According to Dr. Kissebah, the increase in number of fat cells in lower body obese women may be the reason why so many dieters are frustrated in attempting to lose weight below the waist. Obesity occurs by increasing the size of number of fat cells, he explains. While the size of fat cells in the upper body obese can be reduced by dieting, it is extremely difficult, if not impossible, to reduce the number of fat cells below the waist.

Dr. Kissebah is also concerned that persistent dieting by women with lower body fat may expose them to a health hazard as the large number of fat cells below the waist may continue to steal energy from muscle tissue, including the heart.

"It is very distressing for these women to follow a strict diet for months, only to look like two different women joined at the waist. From the waist up she is slender, from the waist down she looks like the diet never started," Dr. Kissebah says.

Dr. Kissebah believes that hormones or genetic factors may contribute to the enlargement of fat cells in the upper body segment and to the elevated blood levels of fatty acid, glucose and insulin.

While men and women have both male and female hormones, some women have more male hormones or are more responsive to these hormones than other women. This could encourage the fat to be deposited in a manner similar to males and may boost the incidence of diabetes in these women.

Researchers are also looking at possible genetic factors. A great deal more study is still required.

In the meantime, the researchers encourage doctors to be conscious of the high risk of diabetes among upper body obese women, check them thoroughly for signs of the disease, and persuade them to lose weight.

Researchers collaborating with Dr. Kissebah are Ronald K. Kalkhoff, M.D.; Nadarajen Vydelingum, Ph.D.; Robert Murray, M.D.; Arthur J. Hartz, Ph.D.; David J. Evans, M.D.; Peter Adams, M.D.; and Alfred A. Rimm, Ph.D.

The Medical College of Wisconsin is the private, independent school for medical and related health sciences on the campus of the Milwaukee Regional Medical Center. It provides medical science education, research, and patient care services. ■

Two Studies Have Good News For Pill Users

Maureen Gardner

The list of health risks associated with oral contraceptives does not automatically grow longer with each new study performed: two recent projects supported by the National Institute of Child Health and Human Development (NICHD) have some good news to offer.

Data from one of the projects, the Women's Health Study, confirmed that the pill protects many of its users from a common and potentially serious gynecological ailment, pelvic inflammatory disease. The other project, a large study conducted in Israel, established that oral contraceptives do not jeopardize the ability of former users to have normal pregnancies and deliveries.

Drawing on data from 16 hospitals in nine U.S. cities, the Women's Health Study verified that women using the pill have a significantly reduced risk of contracting pelvic inflammatory disease (PID). A bacterial infection that affects an estimated 850,000 women in the U.S. each year, PID can lead to infertility or, in very rare cases, death.

The finding on the pill was actually a sidelight of the Women's Health Study, which was a large-scale evaluation of the side effects of another form of birth control, the IUD. One question addressed was whether IUD users have a higher risk of PID than women using the pill, barrier methods, other methods, or no contraceptives at all.

By examining the case histories of 1400 hospitalized PID patients and 3400 control subjects, researchers found that IUD users without a history of PID were twice as likely to contract the disease, compared with women using no form of birth control. Pill users, by contrast, had half the risk of women not using contraceptives.

Those who used the diaphragm or condom also had a reduced risk of PID, because bacteria do not readily cross these barrier devices. The exact method by which the pill protects from PID has not been determined.

The portion of the Women's Health Study data specifically related to the pill and PID was further analyzed by Dr. George Rubin, an epidemiologist for the Centers for Disease Control. In a presentation to the CDC's Epidemic Intelligence Service Conference in April 1981, Dr. Rubin reaffirmed the pill's protective action against PID, stating that it "may be one of the most important non-contraceptive benefits of oral contraceptive use." Dr. Rubin estimates that the pill prevents tens of thousands of PID cases yearly.

The other good news on the pill came from the Jerusalem-based study which found that, in general, women who used the pill before becoming pregnant have no increased risk of pregnancy complications. Investigators from the Hadassah University Medical School compared pregnancy and delivery complications in 2900 former pill users and 13.600 women who never used the pill. With one exception, there were no major differences between users and non-users.

The exception was in former pill users who were pregnant for the first time. This group of women had three times the expected rate of cervicitis, an inflammation of the cervix, or entrance of the uterus. Cervicitis is a benign condition that has been linked in earlier studies with oral contraceptive use.

All in all, the investigators state that oral contraceptive users can anticipate about the same frequency of complications of pregnancy and delivery as women who have used other, or no, methods of contraception. ■

NINCDS Grantee Finds Extensive Spinal Regeneration in Squirrels

Diane Striar

Victims of spinal cord injury—some 10,000 last year in the U.S. alone—may spend their lives yearning for restored mobility. Recent research conducted by NINCDS grantee—and former Institute scientist—Dr. Lloyd Guth offers hope for paraplegics by providing new clues about how damaged nerve cells in the central nervous system can regenerate.

Dr. Guth's study of spinal cord injury in hibernating ground squirrels reveals evidence of extensive regeneration of spinal axons—the nerve fibers that serve as essential link-ups to other nerve cells. Previous studies on nonhibernating mammals have found only minimal regeneration of these axons following spinal cord injury.

Dr. Guth, whose work is also supported by the Paralyzed Veterans of America, chose to study the hibernating squirrel because its sleeplike condition allows the animal to maintain the capacity for nerve cells to regenerate while slowing the formation of excess tissue protein or collagen. Collagen formation, along with tissue death and the growth of cysts at the site of injury, has been considered a possible reason why spinal cord nerve cells do not regenerate completely.

In the experiment conducted at the University of Maryland, Dr. Guth and a team of scientists severed the spinal cords of 30 hibernating ground squirrels and 12 nonhibernating ground squirrels that served as controls. Autopsies performed 1 to 6 months after the operation revealed that the two groups of animals responded differently both to the injury and to the challenge of regeneration.

The nonhibernating squirrels had a dense collagenous scar, extensive formation of cysts and cavities, and showed little evidence that the axons had regenerated. By contrast, the hibernating squirrels had no cysts or cavities within the spinal cord, and the lesion site was composed of a loose, healthy meshwork of cells.

Dr. Guth found that in the hibernating animals a great number of spinal cord nerve fibers had regrown to the margin of the lesion.

"It looks as if these fibers are regenerating, because they have many of the characteristics—the varicosities and growth cones—of regenerating fibers," said Dr. Guth.

Squirrels have proven to be good animal models for studying regeneration of nerve cells. The sleeplike state of hibernation allows the animals to regenerate nerve tissue probably by slowing down the formation of excess tissue protein at the site of injury. NIH Photo 17

However, the regrowing nerve fibers did not enter the lesion site. Instead, upon reaching the margin of the lesion, they turned and ran at right angles to the injured area.

Dr. Guth speculates that changes in the cellular structure of the wound site stop the regenerative process in its tracks somehow—perhaps because the cells cannot provide a proper life support system for nerve fibers.

In a paper published in the December 1 issue of the *Journal of Comparative Neurology*, Dr. Guth suggested that in animals that have a backbone, regeneration of spinal axons requires those axons to interact with other types of cells that surround or support nervous tissue.

"Since the wound tissue at the site of spinal transection in the hibernating squirrel was composed primarily of macrophages, fibroblasts, and vascular elements [cells that make up blood vessels and fibrous tissue]," he wrote, "it is not surprising that the regenerating nerve fibers refused to enter the lesion and preferred to grow along a terrain containing neuronal and glial components." Glial cells provide a supporting structure for nervous tissue.

"The problem that confronts scientists now," Dr. Guth said at a recent NINCDS National Advisory Council meeting, "is to replace the pathological tissue at the site of injury with tissue that might provide factors essential to regeneration. We have to provide a proper environment—one that will guide the neurons across the lesion."

In related research, investigators have successfully transplanted spinal cord tissue from a fetal animal into the same animal's brain. The nerve cells in these grafts continue to develop, mature, and differentiate.

"Transplantation studies," concluded Dr. Guth, "are among the most promising approaches in the field of experimental paraplegia research."

These studies now make up a major portion of the research program at the University of Maryland's Laboratory of Paraplegia Research (in the Anatomy Department). This work is being supervised jointly by Dr. Guth and another NINCDS grantee, Dr. Paul J. Reier.

Dr. Guth formerly headed the Section on Neural Development and Regeneration in NINCDS's Laboratory of Neurochemistry, and is currently professor and chairman of the Department of Anatom at the University of Maryland School o Medicine. ■

Photo compliments of the Bureau of Sport Fisheries and Wildlife

Excess of Malignant Melanoma Found in Nuclear Lab Workers

Helen Ou

Researchers—supported by the National Cancer Institute—have found an excess number of cases of malignant melanoma (MM) of the skin in workers from a nuclear research facility in the San Francisco area. The finding has led to a study to try to identify some of the possible factors responsible for this excess.

The incidence and mortality rates for MM appear to be rising worldwide and significant increases have been noted in the San Francisco area. The disease is often associated with extensive sun exposure, and studies have shown that this type of cancer is more common in the white population and in people in a higher socioeconomic status.

Researchers from the Lawrence Livermore National Laboratory (LLNL) and the California Department of Health Services found 19 cases of MM among approximately 5100 LLNL workers from 1972 to 1977. This number was significantly higher than that expected in a comparable population in a nearby community. The excess MM cases seemed to occur only among laboratory workers and not among the surrounding community, which suggests that an occupational factor may be responsible. Few occupations have, thus far, been associated with an elevated risk of MM.

Of the 19 cases, 17 were men and two were women. Only three cases included workers who were 39 years old or younger, while 11 of the 19 cases occurred in the 40 to 49 age group.

A statistical analysis of observed and expected incidence of MM was highly significant for males, with the observed incidence being approximately three times higher than expected.

MM cases were obtained through computerized records from LLNL employee files and the California Tumor Registry (CTR), a population-based cancer registry for five counties in the San Francisco area.

By matching each of the 19 cases of MM with four comparison workers, the researchers were able to assess whether the MM risk was related to duration of employment, to exposure to any of the various forms of radiation, or to job classification. The data suggested that MM risk was not associated with length of employment nor with radiation exposure. The comparison workers' duration of employment and radiation exposure were not significantly different from those of MM cases.

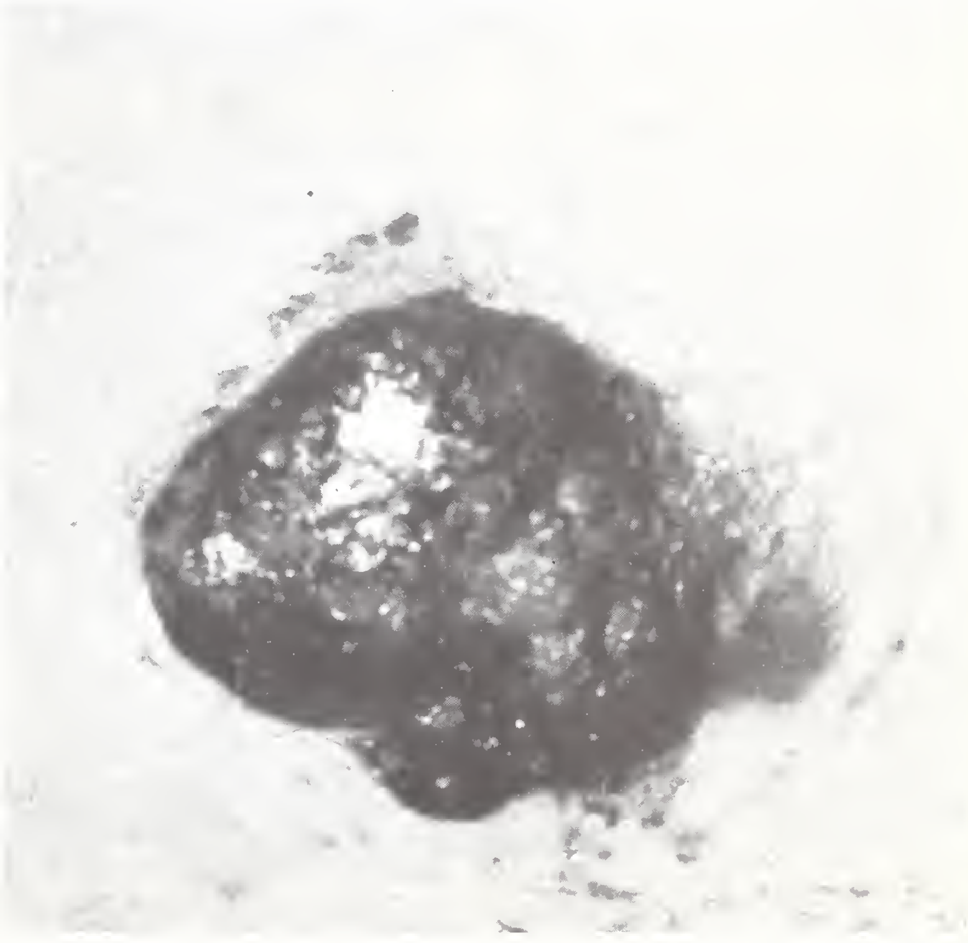
Since 10 of the 19 cases and 31 of the 76 comparison workers were working as scientists, there was no evidence that MM incidence was related to this classification. However, the data did suggest that MM incidence may be higher than expected in chemists. Four of the 19 cases were chemists, compared to 3 of the 76 comparison subjects.

The researchers concluded that LLNL workers had a three-fold excess of MM during the study period. In addition, they also recognized that there were possible sources of bias, which could have explained this excess. For instance, the professional and socioeconomic status of these workers may have influenced them to receive medical care earlier, resulting in more of the cases being reported earlier than those in the comparison population. Another possible bias would result if the proportion of low-risk Hispanics in the study group was different from that in the comparison population.

In addition, MM is unique among cancers in that diagnosis and treatment is sometimes done in a private physician's office and not all cases may be reported to the CTR. However, this does not appear to be a serious problem since potential underreporting would presumably be as great for LLNL workers as for non-employees.

The researchers also acknowledged the possibility of a potential MM hazard in the LLNL environment. They pointed out that, although different sources of bias could account for some of these findings, none of the sources of bias mentioned could alone account for all their results. However, they said that the limited findings to date do not preclude the possibility that the excess MM may be a function of socioeconomic factors and lifestyle, rather than a specific hazard in the work place.

This research was reported in the October 3, 1981 issue of *The Lancet*. ■



A malignant melanoma tumor on the midback of a patient. NIH Photo 1757

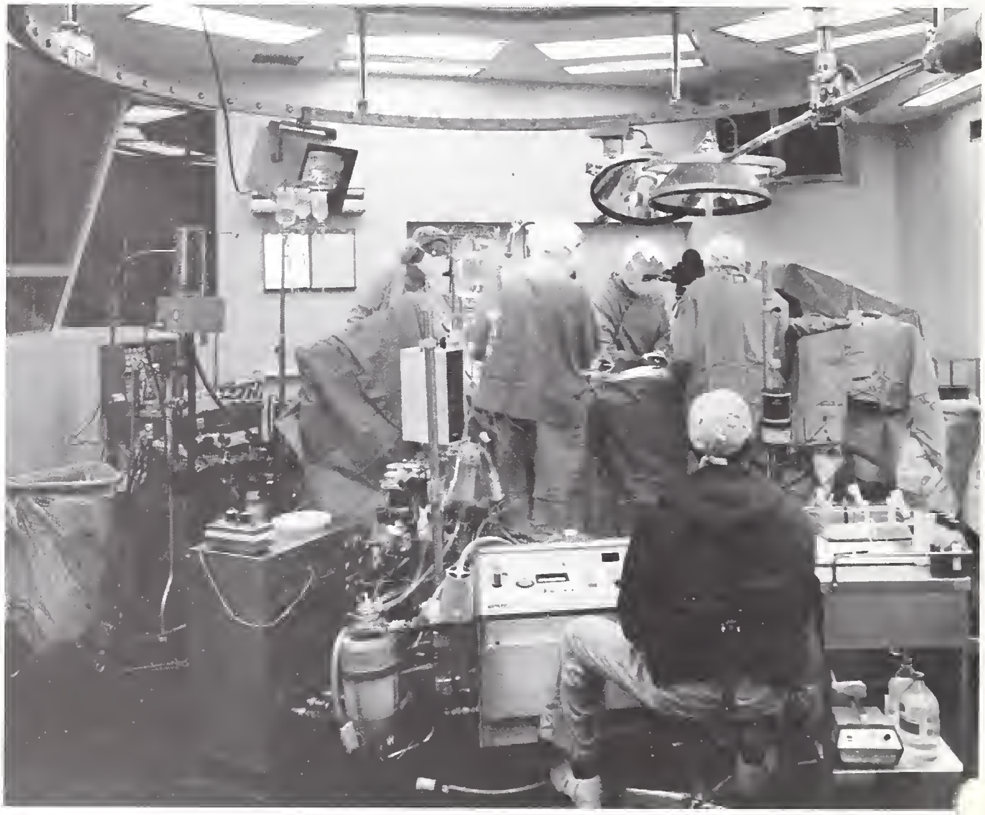
Drama and Technology in the Surgical Suite—A Pictorial View of a Coronary Bypass Operation

Dale Blumenthal

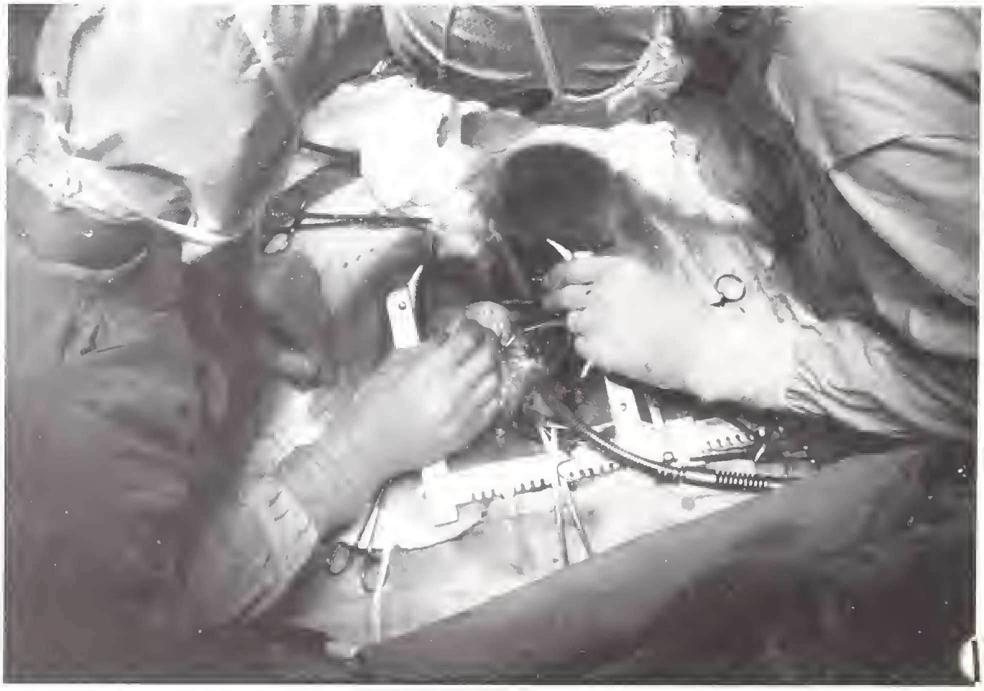
These photographs show a coronary bypass operation, performed by surgeons at the National Institutes of Health. This operation is often effective in controlling angina, the chest pain that develops when the heart muscle is not getting enough blood, and it has been shown to extend life for some types of coronary artery diseases. During the procedure, shown here doctors remove a saphenous vein from the patient's leg. They graft one end to the aorta and the other to a coronary artery, below its narrowed portion. In this way, they bypass the narrowed portion by detouring blood around it.

The number of coronary bypass operations performed last year was nearly 110 thousand. The surgical risks diminish as the procedure becomes more "routine." However, since the procedure is expensive and physically demanding of the patient, the National Institutes of Health is currently sponsoring clinical trials to establish the benefits and indications for this procedure.

The pictures below are recent additions to the Audiovisual Branch, National Institutes of Health picture files. The Audiovisual Branch of the Office of Communications uses slides and photographs to inform the public about developments in health research and about NIH activities. If you have illustrations to share of your own work, or if you would like to borrow photographs, please contact Dale Blumenthal, AV Branch, Office of Communications, Building 31, Room 2B37, NIH, Bethesda, MD, 20205, (telephone 301/496-5895). ■



This operating room shot shows the entire operating team and their equipment. In the foreground is the heart-lung machine. At left, behind the patient's head, is anesthesia equipment. Above the lamps, at top right, is a monitor which gives a continuous read-out of respiration rate, blood pressure, heart rate. NIH Photo 1760



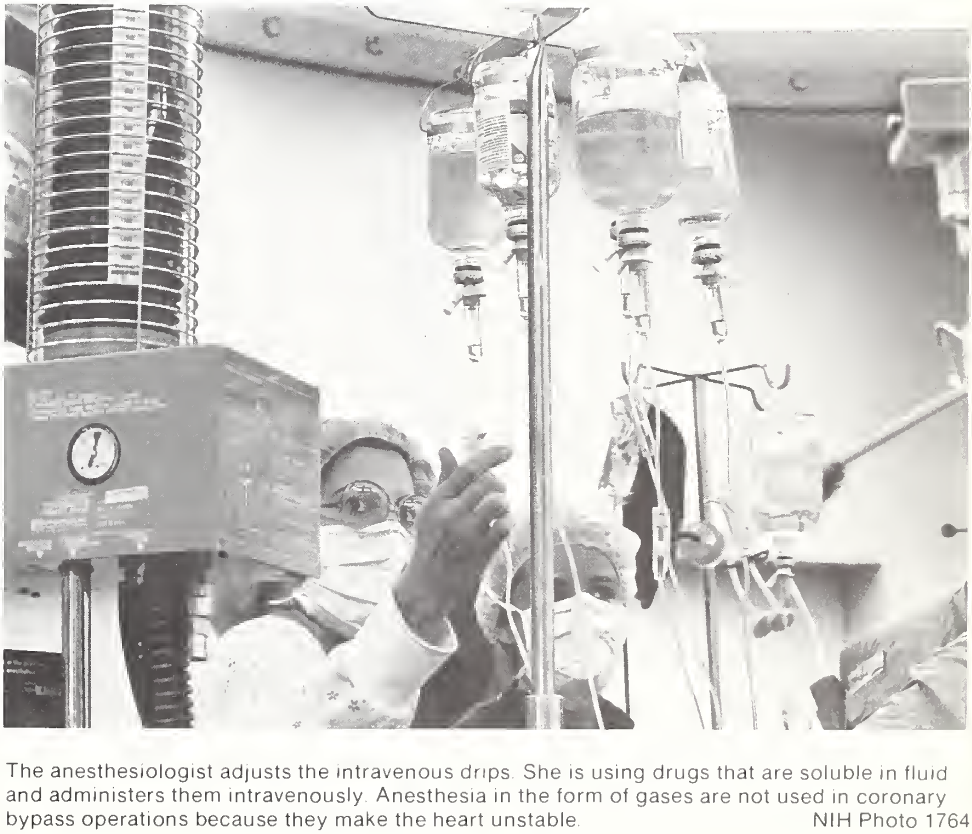
Doctors use a rib retractor to keep the chest wall separated as they work on the patient's heart. Blood flows from the heart to a heart-lung machine and back through two lines, one of which is prominent in the lower right-hand side of the photograph. NIH Photo 1761

Photos taken at the National Heart, Lu
and Blood Institute's surgical suite at t
NIH Clinical Center.

This overview of a coronary bypass operation shows the surgical team at work. Two members remove a saphenous vein from the patient's leg while two others open the patient's heart. In the upper right of the photograph, a technician monitors the heart-lung machine NIH Photo 1762

A perfusion technician prepares the heart-lung machine, which will assume the function of the heart and lungs during the operation. NIH Photo 1763

Photos by Jerry Hecht



The anesthesiologist adjusts the intravenous drips. She is using drugs that are soluble in fluid and administers them intravenously. Anesthesia in the form of gases are not used in coronary bypass operations because they make the heart unstable. NIH Photo 1764

Cancer Risk Following Medical Irradiation Outlined by NCI Epidemiologist

Aubin Tyler

Studies of populations exposed to radiation as part of medical diagnosis or treatment—from the 1930's to the present—are providing clues to scientists about the effects of radiation on cancer risk and on the origins of cancer in general. A paper by National Cancer Institute epidemiologist John D. Boice, entitled "Cancer Following Medical Irradiation" summarizes the most important of these studies and arrives at some basic conclusions about radiation-induced cancer:

- A single radiation exposure may increase the risk of cancer.
- Radiation-induced cancers cannot be distinguished from normally occurring cancers—thus making risk assessment difficult.
- All cancers appear to increase after irradiation except chronic lymphocyte leukemia (CLL), and possibly Hodgkin's disease and cervical cancer.
- Body organs have different sensitivities to the effects of radiation. Breast, thyroid and bone marrow tissue appear to be the most sensitive while that of the kidney, bladder and ovary seem to be the least.
- The percent increase in cancer incidence per rad of exposure is not the same for all types of cancers.
- Leukemia, except CLL, appears to be the most common radiation-induced cancer.
- Other than the dose of radiation, age at exposure may be the most important determinant of future cancer risk and this age-related risk probably varies for different types of cancers.

Medical treatment of noncancerous disease with X-rays, a practice that is generally unacceptable today, was more common 40 and 50 years ago, before the hazards of radiation exposure were known. For some diseases, radiation was the best or the only treatment available.

Between 1935 and 1954, more than 14,000 patients were treated in British radiotherapy clinics for a rheumatoid condition of the spine known as ankylosing spondylitis. This is a chronic progressive form of arthritis that causes inflammation and eventual immobility of the joints, primarily of the spine. Evaluation of the death rates of these patients in the 1960s showed higher than expected rates of leukemia.

Instead of the five or six cases of leukemia expected from natural causes other than radiation, 52 persons developed the disease following an averge radiation dose of 370 rads to bone marrow. Other excess cancers of heavily irradiated organs were found to occur about 10 years after exposure and included cancers of the lung, pharynx, stomach, pancreas, bone, and lymphoma.

Because all influencing factors cannot be accounted for in such a study, scientists wondered if something in addition to X-rays could have been responsible for the higher cancer rates. The fact that this was an already ill population, for example, might have predisposed them to cancer. However, a 1977 study of British patients with the same rheumatoid condition who were not treated with X-rays failed to find evidence of abnormally high cancer rates. The results suggested that excess cancers occurred in the earlier group of patients primarily as a result of their X-ray treatment, rather than other risk factors.

Increased cancer rates have also been observed in women given radiotherapy for postpartum mastitis, a staphylococcal infection of the breast, that is common in nursing mothers. From 1940 to 1955, hundreds of women in Rochester, New York, were treated for the breast inflammation with an average radiation dose of 247 rads. A 1977 mail survey found that among 571 of these women, the breast cancer rate was twice that of a nonirradiated comparison group. Since women who did not receive X-ray treatment were used for comparison, "it seems unlikely," said Boice, "that causes other than X-ray exposure were involved in the higher cancer rates."

In the 1930s and 40s, radiation was a fairly common therapy for childhood conditions such as ringworm of the scalp, enlargement of the thymus gland, tonsils and adenoids, and acne. But by 1975 it was evident that people exposed to these radiation treatments had a significantly higher incidence of thyroid cancer that developed five to 35 years after exposure.

That year, a mail survey of 2,872 children irradiated in infancy for enlarged thymus glands, found a rate of thyroid cancers that was much higher than the norm: 24 observed compared to 0.29 expected. There was also a higher incidence of radiation-induced thyroid cancer in females and among Jews. No thyroid cancers occurred in untreated siblings.

The onset of thyroid cancers showed a sharp rise during adolescence, leading investigators to believe that thyroid stimulating hormone (TSH) may play a promoting or secondary role in the development of radiation-induced thyroid cancer. Additionally, children may be more susceptible than adults to radiation-induced thyroid cancer, particularly if rapidly dividing cells—like those of the thyroid gland childhood—are more prone to develop abnormally after radiation injury than less active cells.

In 1977 the NCI launched a public education campaign to alert persons who received X-ray therapy to the head and neck during the childhood that they may be at high risk of developing thyroid cancer. The NCI recommended that these persons, as well as anyone with a lump in the neck or throat area, consult a physician and receive regular thyroid exams.

Meanwhile, an international study designed to estimate the risk of second cancers from X-ray treatment of malignant disease turned up some very paradoxical results. The 10-year study annually followed 30,000 women who were treated for cervical cancer with radiation in nine different countries in the late 1950's and early 1960's. Although X-ray doses to the pelvic bone marrow had been enormous (averaging 500-1500 rads), no excess leukemia was found.

"The results were surprising," Boice said, "we expected 50 to 60 excess (radiation-induced) cases."

The results were particularly odd because of the known leukemia risk associated with much lower doses of radiation to the pelvis, at one time used to treat metropathia hemmorrhagica, or uterine hemorrhage. A 1976 study of 2,068 women treated 10 years earlier with X-ray doses of 500 to 1000 rads to their ovaries and an average pelvic bone marrow dose of 134 rads, found seven cases of leukemia, instead of the expected two or three.

Boice suggested that perhaps the doses given to the women with cervical cancer were so massive that portions of their bone marrow cells were killed and thus unable to divide, or become cancerous. A lower than expected rate of chronic lymphocytic leukemia (CLL) was also reported among cervical cancer patients who received X-ray treatment; the type of lymphocyte, or white blood cell, involved in the disease is especially sensitive to radiation cell killing and this phenomena may possibly explain the observed deficit. (In fact, excess cases of CLL have never been reported in any radiation study.) The study of irradiated cervical cancer patients has now been expanded to 200,000 women and will examine secondary cancers other than leukemia that may be related to X-ray therapy for cancer.

The present use of low dose radiation for diagnosis raises other questions about radiation and cancer risk. For example, did the use of prenatal X-rays (averaging one to 10 rads) contribute to the development of childhood cancers? Controversy over this issue began with the findings that Japanese A-bomb survivors, exposed to the radiation blast *in utero*, failed to develop the amount of radiation-induced cancers predicted from studies of children who experienced prenatal X-ray exposure. Many studies of childhood cancer have found an association between prenatal X-rays and cancer risk; however, the absence of a risk in atomic bomb survivors suggests that some selection of children destined to develop cancer might have occurred.

"The fact that prenatal X-rays are needed at all could be an indicator of future cancer risk due to poor fetal or maternal health rather than X-ray exposure," Boice said.

Because of the increasing use of abdominal ultrasound for fetal monitoring, prenatal exposure to low dose radiation may no longer be a major public health issue, but it is a radiation-biology issue, according to Boice. He is not studying a large population of twins to examine the question further. As a group, twins are often exposed to X-rays in the womb, although they are not necessarily sick, simply because their mothers are larger than women carrying one child. Boice thinks the data from this study may be good indicators of cancer risk from prenatal X-rays.

"In general," he said, "the lower the dose, the lower the hazard. But we live in a sea of low dose radioactivity and our species has managed to survive. Each year we get about 0.1 rads of radiation exposure from cosmic rays and naturally occurring radionuclides in the environment. In 70 years that amounts to seven rads of exposure."

What is needed, according to Boice, is a systematic theory to predict the risk for radiation-induced cancers in the low dose range. Additional studies to evaluate the effects of low and high dose radiation exposure, multiple and single exposures, age at exposure, and the interaction of radiation with other carcinogens may help to accomplish this goal as well as provide insights into the general principles of cancer induction. ■

Radiation therapy is used to treat a variety of cancers, and has been used more extensively in the past to treat noncancerous diseases. Studies of people exposed to radiation for medical treatment and diagnosis are providing investigators with important facts about the increased risk of cancer from radiation and the development of cancer. NIH Photo 1758

Researchers Study Mutant Mice for Clues on Human Dwarfism

Hilah Thomas

Scientists at the National Institute of Dental Research (NIDR) report that hereditary, molecular variations in the cartilage of inbred, mutant mice explain differences between several types of dwarf mice. These mice are promising models for the study and possible explanation of some abnormalities of human skeletal growth.

Dr. Kenneth S. Brown, a geneticist in the NIDR Laboratory of Developmental Biology and Anomalies, notes that some form of dwarfism appears in about one of every thousand newborn humans. The hereditary syndromes of many dwarfs include an incidence of cleft palate and other craniofacial malformations that is higher than that found in the general population. At present, the underlying causes of these human syndromes are unknown, but most are thought to result from single defects in synthesis of cartilage. Bone growth and form in the spine and limbs follow cartilage development, so if the cartilage is defective, the bone will be also.

Perhaps the most common type of human dwarfism is called achondroplasia, meaning cartilage growth deficiency. Individuals with this disorder have a large head and a normal-sized trunk, but short arms and legs. Another type of dwarf is pseudoachondroplastic spondyloepiphyseal dysplasia (SED), meaning a skeletal defect resulting from an ochondeoplasia like deficiency of cartilage. These individuals appear normal at birth, their heads develop normally, but by age two their bodies are small and their limbs are very short. In a more severe type of dwarfism (achondrogenesis—failure of cartilage to develop) considerably less than the normal amount of cartilage is present at birth. These babies are uniformly small, most have cleft palates, and death occurs in infancy. Another severe syndrome is thanatophoric (death causing) dwarfism in which the head is large, the eyes far apart, and the nose saddle-shaped. The short limbs stand out from the body instead of reaching down, and life expectancy is brief. Finally, in Lowe's Syndrome growth failure is accompanied by bone, kidney, and eye diseases as well as mental retardation.

Dr. Brown's team has studied mutant mice with defects that appear to resemble these human conditions in a number of ways. Mice with the defect called disproportionate micromelia (Dmm), meaning unevenly shortened limbs, appeared in a laboratory strain of mice exposed to radiation. Newborn animals carrying this gene from only one parent (heterozygous) look normal at birth, except that 10 percent die with cleft palate. Adult survivors are obvious dwarfs with limbs proportionally shorter than their bodies.

Mice carrying the Dmm gene from each parent (homozygous) are born dead or die soon after birth. They all have broad heads, short snouts, protruding tongues and cleft palates. Their bodies, though small, are not as dwarfed as their limbs. The limb bones nearest the trunk are more seriously dwarfed than those nearest the paws. These animals may be models for achondroplastic and SED types of human dwarfism.

The other type of dwarf mouse suffers from cartilage-matrix deficiency (cmd) that results from a spontaneous, recessive, non-sex-linked mutation. Heterozygous mice with one cmd gene look normal. The homozygous mice with two cmd genes have little matrix material between the cartilage cells and die soon after birth or are born dead. In appearance they differ from the homozygous Dmm mice in that the long bones as well as the body are evenly dwarfed. However, they resemble these Dmm mice in having cleft palates and misshapen heads. The pups can easily be mistaken for each other. The cmd defect may be a model for the achondrogenic and thanatophoric types of human dwarfism.

Comparison of the long bones of the two types of homozygous mice showed that cmd bones have normal diameters but all are proportionally short. The same bones in the Dmm mice have larger than normal diameters, and are unequally shortened.

The scientists cultured the epiphyses (growth centers) of the fetal long bones of both kinds of mice with radioactive chemicals that are always incorporated into the proteoglycans of cartilage matrix. Proteoglycans are large molecules with a protein core to which various complex carbohydrates are attached. Another important protein molecule in the matrix produced by cartilage cells is a special type of collagen (type II) that gives cartilage much of its strength.

Analysis of matrix chemicals showed a number of differences between the two mutant mice. For example, the proteoglycan in the homozygous Dmm mouse was chemically normal but reduced in quantity, whereas that of the homozygous cmd mouse was chemically different from the proteoglycan characteristic of normal cartilage matrix and also reduced in quantity.

From their studies the investigators conclude that the cmd mouse fails to synthesize the core protein of its proteoglycan and therefore has a smaller volume of matrix around the cartilage cells. These cells also produce 20 percent less collagen than normal mice, but the cartilage has normal strength. In contrast, the matrix volume of the Dmm mouse cartilage is not reduced as much as in cmd, but the matrix is weak. The collagen in Dmm is reduced 30 percent and is localized around the cells, while in cmd, matrix collagen is normally distributed. This mouse synthesizes somewhat less proteoglycan than normal mice, but the amount of matrix is not as reduced as that of the cmd mouse and the structure is normal.

Clearly the Dmm gene results in defective collagen synthesis, whereas the cmd gene results in defective proteoglycan. This is the first time that such specific molecular mechanisms of dwarfism have been identified.

In children with the rare Lowe's syndrome, Japanese scientists have recently reported finding a defect in the incorporation of sulfur into certain proteoglycans. Although this type of dwarfism is not exactly duplicated in mice, nevertheless Dr. Brown points out that the (bm) mouse gene called brachymorphic, meaning short-form, results in similar biochemical defects that may prove helpful for studying this human problem.

The brachymorphic mouse is recognized only when it has two recessive bm genes. These mice are fertile and live a normal span of time but are prone to malocclusion from short upper jaws. Their cartilage has normal strength, but the amount of matrix is reduced especially in the epiphyses of the limb bones. Their growth plates do not synthesize enough of the molecule that adds sulfur to the glycosaminoglycans which are necessary for forming cartilage matrix. ■

Scientists Meet at Leeds Castle, England for Summit Conference on Leukemias and Lymphomas

Patricia Newman

The first scientific summit conference ever held by the United States and Great Britain recently concluded at Leeds Castle, in Kent, England. Twenty-four prominent experts on leukemias and lymphomas met to discuss the most promising leads in research on these diseases and ways to intensify collaborative research between scientists in the two countries.

The meeting resulted in a review of significant research results from scientific disciplines as varied as medicine, genetics, and the rapidly advancing fields of cellular and molecular biology. A scientific summary of the meeting is expected to be published in *Nature*. However, the following brief summary provides an indication of what progress has occurred in recent years, and which research approaches appear most promising.

The following perspectives emerged:

- Specific changes in cell chromosomes are a reliable indicator of cancer for chronic myelocytic leukemia, acute myelogenous leukemia, acute promyelocytic leukemia, and Burkitt's lymphoma, and are suggestive of leukemia or lymphoma in patients with an hereditary muscular disorder ataxia telangiectasia, who have an increased risk of cancer.
- Scientists can now isolate human chromosomes and order their genes. It now appears possible that they soon will be able to identify the molecular sites of specific translocations (from the normal site on the chromosome to a new site on the same or another chromosome). In coming years, research in molecular biology is expected to identify more closely the molecular abnormality that is leukemia.
- Each patient with leukemia or lymphoma has many abnormal cells circulating in his blood or lymph system; only a small percentage of these cells may give rise to new cancer cells. Treatment might be improved by finding ways to direct drugs against the true immortal cells.
- No two leukemic patients appear to have the same marker antigens on the leukemic cells, or the same requirements for growth of their cells in laboratory cultures.
- Defects of DNA repair have been identified in Downs' and Bloom's syndromes and in Fanconi's anemia. Patients with these hereditary conditions have an increased risk of leukemia or lymphoma, as do patients with certain types of immune system disturbances.
- A number of chemicals, such as alkylating agents, are very effective in treating existing cancer, but also increase surviving patients' risk of developing leukemia.
- Drug resistance is a major cause of treatment failure. A new mathematical model to explain the rate of development of drug-resistant cancer cells has been published. The model has stimulated studies of alternating, non-overlapping drug regimens that can kill cancer cells before they develop resistance.
- Because of the risk of medical complications following bone marrow transplantation, this infrequently used treatment is expected to have èven more limited applications as less toxic therapies are developed.
- Animal studies suggest that it may be possible to develop drugs that will help leukemic cells return to a normal appearance and function. (It is far too early to know whether such drugs will ever supplant conventional drug therapy.)

Cosponsored by the National Cancer Institute and the Leukaemia Research Fund of Great Britain, the meeting was recognized by Prime Minister Margaret Thatcher of Great Britain, and by President Ronald Reagan and Vice President George Bush of the United States.

In a message to conference participants, President Reagan said "...This joint endeavor represents an extraordinary effort by a dedicated group of experts from both countries to address the complex issues confronting current research in leukemia. As one of the most common forms of cancer striking young children, leukemia is a particularly heart-rending disease. All progress in treating this illness represents a major contribution to the humanitarian concern and well-being of all people..."

The conference participants discussed possible U.S. Great Britain collaborative projects, including studies of a unique virus isolated in the United States from a human T-cell lymphoma by Dr. Robert C. Gallo and his coworkers at the National Cancer Institute. A similar, if not identical, virus was isolated again in Japan recently, where leukemias and lymphomas of mature T-cells are more common than in the United States.

On the molecular level, there is now clear evidence from animal models that a virus that causes leukemia after a long latent period does so by activating certain cellular genes. Participants discussed the implications of these findings for other forms of cancer, and agreed that the eventual merger of chromosomal and molecular studies will provide insights into the causes of cancer.

Another important model for human cancer, discussed at Leeds Castle, is the Epstein-Barr virus, which causes leukemias and lymphomas in sub-human primates. This herpesvirus is associated with Burkitt's lymphoma and with a very rare, acute lymphoproliferative disease in children.

Leeds Castle has existed since the ninth century. Its last private owner, Lady Baillie (whose mother was American), bought the castle in 1926 and established the Leeds Castle Foundation as a charitable trust. The Foundation sponsors medical research seminars for leading scientists and physicians from around the world.

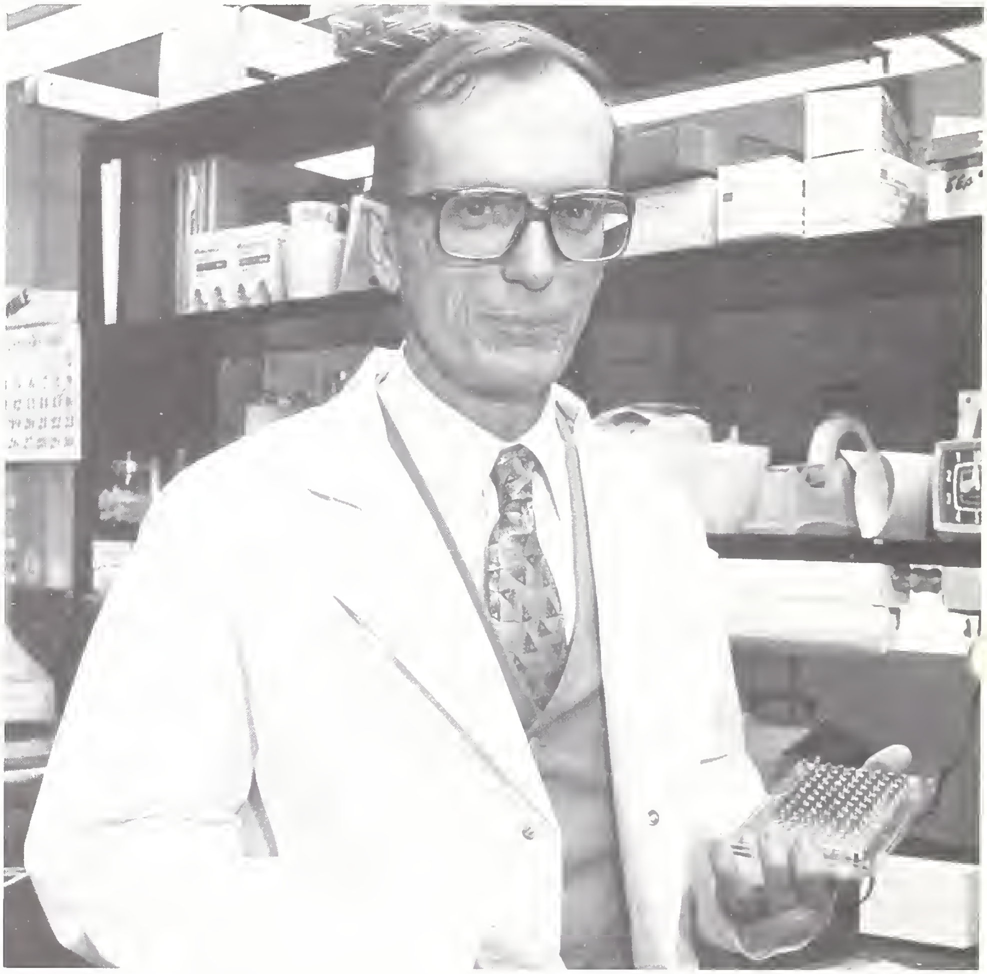
Co-chairmen of the summit conference on leukemia and lymphoma were: Professor Sir John Dacie, Chairman of the Medical and Scientific Advisory Panel of the Leukaemia Research Fund of Great Britain, and Henry S. Kaplan, M.D., Maureen D'Ambrogio Professor of Radiology and Director of the Cancer Biology Research Laboratory, Stanford University Medical Center, Stanford, California.

The meeting featured round-table discussions of the latest developments in research on the leukemias and lymphomas and identification of research needs. There were no formal presentations of data. Discussions were aimed at challenging preconceived perspectives, and identifying new ways to study these diseases.

Participants agreed to meet in the United States one year from now to examine progress in cellular and molecular research. ■

Saving the Children: Neuroscience Reaps Rewards of Infection Research

Ray Fleming



Dr. John Sever, NINCDS: great expectations for infection research. NIH Photo 1759

Mid-summer 1976. The Nation's newspapers were full of stories about the puzzling, pneumonia-like illness that had struck scores of American Legion conventioneers returning from a Philadelphia meeting. Doctors at that time were unable to explain or treat "Legionnaire's disease," as the illness was dubbed—a dangerous infection that was later found to have threatened children as well as adults. But in a matter of months, after one of the most complex investigations of an epidemic ever undertaken, a previously undiscovered bacterial agent was found to be the cause. Today, the disease can be diagnosed and treated with antibiotics.

The identification and treatment of Legionnaire's disease is one of the more spectacular examples of progress in infectious disease and immunological research during the 1970's—progress that particularly benefitted the world's children. The decade saw the eradication of smallpox and the development in the U.S. of highly successful immunization programs against rubella, measles, and mumps. Methods were developed to diagnose hepatitis B, an achievement that saved thousands of newborns and adults from serious infection. Several different viral groups were identified as causing certain gastrointestinal problems in children.

Infection research has also protected large numbers of youngsters from nervous-system disease. According to Dr. John Sever, Chief of the Infectious Diseases Branch at the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS), the neurological benefits gleaned from a decade's advances in the study of infection are hopeful signs of further progress in the 1980's.

Reviewing research advances of the past 10 years, Dr. Sever points to an unexpected windfall produced by the extensive U.S. immunization program against measles: a drastic reduction in cases of subacute sclerosing panencephalitis (SSPE). This fatal brain disease of children, for which there is no cure, is caused by the lingering measles virus, but measles vaccines have now all but eliminated SSPE.

Other vaccines developed in the 1970's have aided children with nervous system disorders: a pneumococcal vaccine against infant ear infections, a more potent and effective rabies vaccine, new vaccines to protect children from meningococcal infections. Poliomyelitis, notes Dr. Sever, is also well under control, thanks to the continuation of the massive immunization program begun in the 1950's. Today, there are fewer than 10 cases of poliomyelitis in the U.S. each year, and Dr. Sever expects that future occurrences will be even rarer.

The introduction of new antibiotics over the past decade has also had a positive effect on children's neurological problems. Life-threatening disorders such as neonatal meningitis can now be successfully treated, and neuroscientists are encouraged by the success of new "tailor-made" antibiotics against a variety of infections. One of these preparation, for example, is particularly useful against children's ear infections.

Such successes have opened up new opportunities in infectious disease research, says Dr. Sever. Among the unsolved riddles facing scientists is Reye's syndrome, a children's disorder which can result in severe liver damage and sometimes fatal brain damage. Reye's syndrome occasionally follows chicken pox or influenza infections, but little is known about why Reye's develops only in certain children. Better treatment and prevention of this disease will depend heavily on identifying the factors contributing to its development.

The control of Reye's syndrome and other neurological problems could hinge on the development of more effective vaccines. New vaccines for chicken pox and influenza would not only reduce the incidence of the infections themselves, but might lower the number of Reye's cases. A chicken pox vaccine might also prevent shingles, a nervous system disorder caused by the chicken pox virus. An additional need for the 1980's, Dr. Sever believes, is a vaccine for Group B meningococcal bacteria, the agent responsible for the most frequently occurring type of meningitis in the U.S.

Meningitis due to Haemophilis influenzae type B bacteria is a brain infection which attacks nearly 30,000 children in the U.S. each year. The bacteria are resistent to penicillin in approximately 20 percent of the cases, Dr. Sever points out, and better antibiotics and preventive vaccines are needed. In addition, cytomegalovirus infections in pregnant women account for over 3,000 brain-damaged newborns yearly in the U.S., a total which immunologists and infectious scientists hope to reduce in the next decade.

According to Dr. Sever, otitis media, a common childhood infection in the middle ear, should also be heavily investigated in the 1980's. Some studies suggest that more information on middle-ear drainage might be helpful in understanding the problem. To encourage research on the problem, NINCDS recently funded a major otitis media research center at the University of Pittsburgh.

Slow virus diseases should receive special research attention in the current decade, according to Dr. Sever. These illnesses, caused by the sudden activity of a virus that may have lain dormant for years, sometimes have their roots in common childhood infections. Progressive rubella panencephalitis and progressive multifocal leukoencephalophy, for example, are both slow virus diseases linked to common viral infections, and both result in severe brain damage. Scientists need to better understand how the early infections are controlled in normal individuals, and why they flare up years later in others.

Successful research in infectious diseases and neuro-immunology is bringing many childhood neurological problems under control. A number of research advances have already been transferred to clinical medicine to help physicians diagnose, treat, and prevent infectious diseases.

"We've been fortunate," says Dr. Sever, "in identifying many infectious agents and controlling the illnesses they cause. But there are still a lot of them we can't identify."

"More research," he concludes, "is obviously needed before we can pinpoint those agents and rid our children of their effects." ■

Package Antibody Tests Deemed Unnecessary, Unreliable

Ray Fleming

Physicians testing women for rubella antibody by using kits claiming to provide "complete antibody profiles" may be doing their patients a disservice.

The widely available kits may initially appear desirable because they can test for four or more different antibodies. But according to Dr. John Sever, Chief of the Infectious Diseases Branch at the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS), the kits can give unreliable results leading to erroneous diagnoses and treatments.

Collectively called TORCH packages after the microbial agents (toxoplasma) rubella, cytomegalovirus, herpes simplex, and others) whose antibodies are being tested, the TORCH tests can also cost over $55, in contrast to the $10 charge for an individual rubella test.

In an article in the November 1981 issue of *Contemporary Obstetrics/Gynecology*, Dr. Sever points out the importance and general reliability of separate rubella tests for verifying the immune status of women, but advises against the routine use of the broader TORCH tests for pregnant patients. Results from TORCH testing are frequently inconclusive, he says, and individual tests are often a better choice when a specific infection is suspected.

The microbial agents whose antibodies are sought in TORCH testing cause important perinatal infections that may seriously damage the fetus or newborn. Infected women usually show no symptoms, or have relatively limited disease. Infants infected in the perinatal period can have mild to severe damage or can die.

Tests offered by clinical laboratories for toxoplasma, cytomegalovirus, or herpes simplex can also be unreliable. Should testing be needed, says Dr. Sever, physicians should seek the services of reference laboratories such as the Centers for Disease Control or the larger state labs. ■

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National Institutes of Health
Building 31, Rm. 2B-10
Bethesda, Maryland 20205

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News&Features from NIH

March 1982

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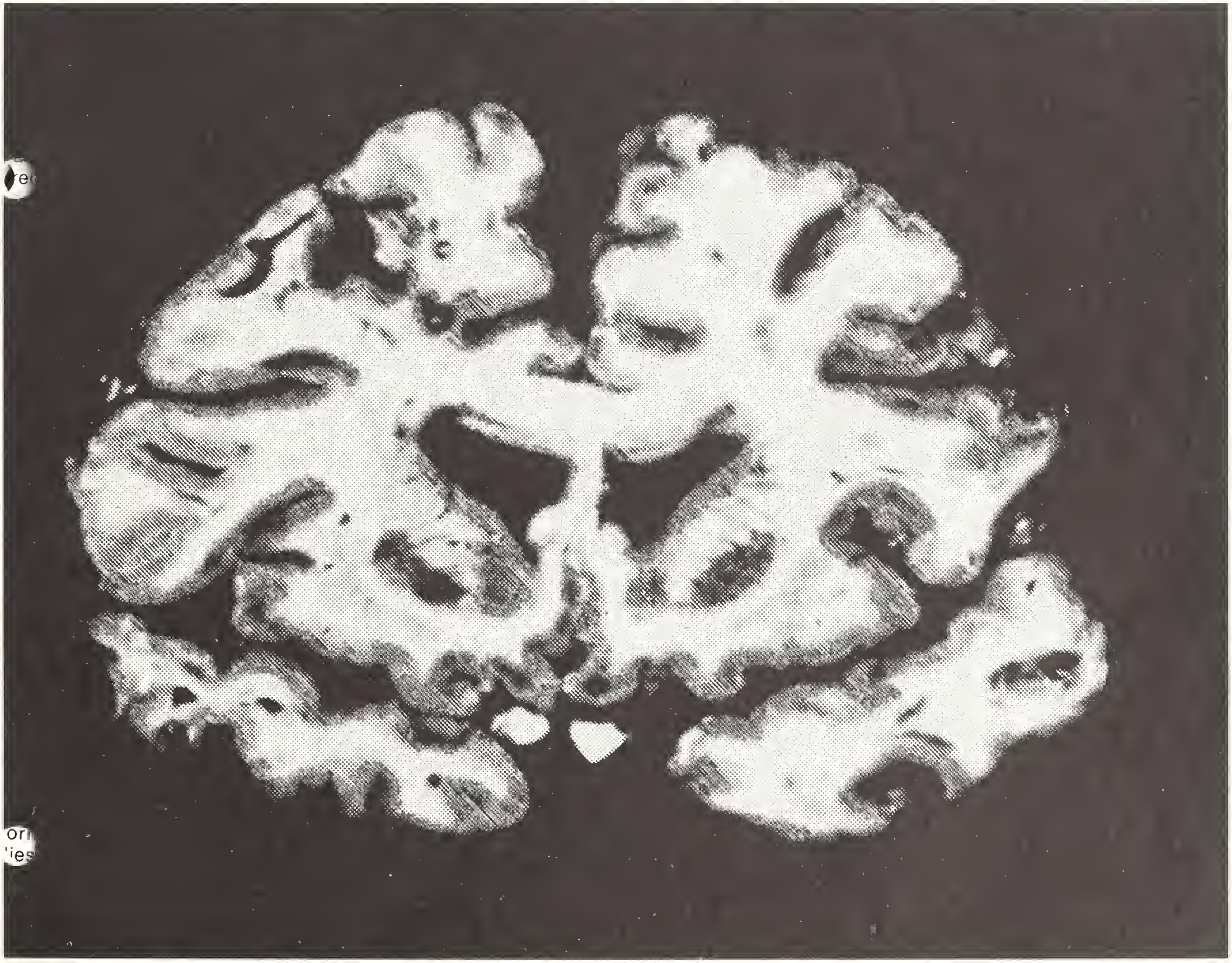

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News&Features
from NIH

March 1982

Brain banks provide a unique and valuable service to researchers studying the brain and neurological disease. Donated brain tissue from patients with neurological problems can help scientists identify the abnormailty responsible for the damage and furnish clues for controlling and preventing the disorder. For instance, certain findings suggest that slow viruses may be the cause of the demyelinating disorders seen in multiple sclerosis. See page 10.

Neuroscientists are finding that opiates manufactured in the brain may have many as yet undiscovered, functions. One clue to this theory is the widespread location of opiate receptors throughout the brain. Using new techniques, scientists are able to map the distribution of receptors, and with this information, obtain insights into the complex normal and abnormal mechanisms of the brain. See page 6.

White blood cells from a patient with a rare type of leukemia have been found to spontaneously produce interferon. This finding-not recognized before-leaves scientists wondering whether the interferon is causing the disease or is one of the body's defenses against it. See page 14.

Transferring genes may make it possible, for the first time, to treat certain hereditary diseases. NIH scientists have succeeded in transferring genes from one animal species to another, which may eventually be applied to man. No only did the genes work properly, but they also were passed to their offspring. See page 4.

One man's personal struggle and victory over Parkinson's disease marks the triumph of the human spirit together with the advances of modern medicine. For 20 years, Sidney Dorros has bravely battled with his disease, trying many different therapies and approaches. Acceptance and a positive attitude are as important to his routine as his daily medications. See page 2.

Editorial Staff

Mary Donovan
Editor
Susan Gerhold
Associate Editor
Helen Ou
Special Assistant
Maureen Napper
Editorial Assistant

NIH News Branch
Building 31, Room 2B-10
Bethesda, Maryland 20205
Phone:
(301) 496-2535

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Parkinson's Disease Patient Conquers His Illness with Modern Medicine and a Positive Attitude

Diane Striar

"Many people think there's a simple magic formula that enables me to cope with Parkinson's disease," says 56-year-old Sidney Dorros, longtime research patient at NIH. "But there is no simple answer—there are 100 answers. If one pill doesn't work, try another."

Practicing this philosophy, the slim and energetic Dorros has tried all the major drug treatments of the past two decades since he developed Parkinson's disease at the unusually young age of 36. He first encountered many of these drugs at the NIH Clinical Center where he was a subject in over 30 studies. He was the first NIH patient to benefit from L-dopa and later, in a study conducted by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS), was one of the first patients in the U.S. to benefit from bromocriptine. This drug was recently approved by the Food and Drug Administration for use in Parkinson's disease.

But Dorros' ability to cope with the uncontrollable tremor and muscular rigidity of Parkinson's disease depends as much on his skill in accommodating to the illness as it does on medication.

In his recently published book **Parkinson's: A Patient's View,** he defines accommodation as "accepting and making the best of one's situation." The process is both physical and psychological and includes adjusting diet, exercise, rest, and recreation to achieve the best conditions possible for medication to work.

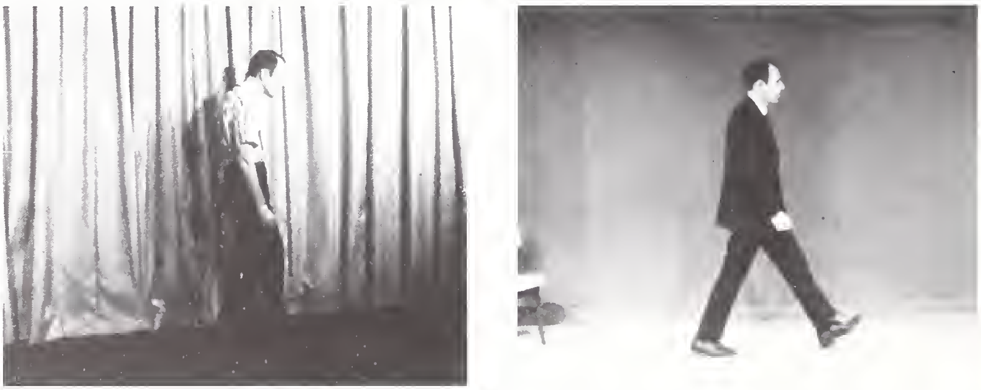
Love and helping others are equally significant principles in Dorros' philosophy of accommodation. They are particularly important in his current roles as newlywed, book promoter, and activist in the Parkinson's self-help movement.

Mr. Dorros married for the second time on January 1 (his first wife died several years ago from cancer). "My New Year's resolution," he says, "is not to forget my wedding anniversary. I wanted to get married January 1 so that I would still remember the anniversary—even if I lost my memory," he jokes. "Although I think my wife had more romantic reasons for a New Year's Day wedding."

Dorros and his new bride, Donna, first met 21 years ago when they both worked at the National Education Association (he as director of publications, she as production coordinator). Although they eventually lost touch, their acquaintanceship was renewed when he called to acknowledge a late condolence note that Donna had written following his first wife's death.

As their friendship advanced to affection and love, Dorros began to feel younger, healthier and happier—prompting relatives and the doctors at the Clinical Center to comment on his improved condition.

The last 3 years in particular have been good ones for Sidney Dorros, who credits his improvement to "love and security. I'm taking the same medication I used to but I can function better," he says.



Sidney Dorros used to walk awkwardly before L-Dopa treatment (left photo). He was the first NIH patient to benefit from this treatment (right photo) and later one of the first patients to benefit from another drug called bromocriptine.

NIH Photo 1769

NIH Photo 1770

His renewed energy is being used not only in an extensive multi-media promotional campaign for his book, but also in work with the Parkinson's Society of Greater Washington, a local self-help group. Dorros also finds time to serve as director of education and training for the Parkinson Support Groups of America. This umbrella organization—still in the formative stage—plans to assist all Parkinson's disease support groups in the U.S.

"We're trying to carry our philosophy to the general public," he says. "I have become a symbol for people by showing them that they can cope. What most impresses people is that I'm not perfect—but that I carry on despite my problems."

Dorros' ability to "carry on" with his busy schedule is aided by his attention to nutrition and exercise. He tries not to eat too much protein at a single meal, for example, as it interferes with the effectiveness of the drugs. He also restricts foods that have a lot of Vitamin B-6 as they too impede the action of anti-parkinsonian drugs.

Dorros' exercise regimen includes swimming, dancing, and walking. "I'm trying to walk the entire length of the C&O Canal," he says. "So far I'm up to 70 miles."

Although Dorros uses his remaining abilities fully, he does not make "futile attempts at what I can't do. I can't handle heavy things and I don't go fishing when I'm apt to get stuck." Getting stuck for a Parkinson's disease patient can mean unusually slow movement (bradykines or inability to move at all (akinesia).

Dorros' mobility will be evaluated in a forthcoming experiment at NIH. "I feel an obligation to continue participating in research," he says. His sense of obligation stems from his appreciation of "the medical research and health care personnel whose discoveries and treatment have made it physically possible for many parkinsonians to enjoy a meaningful life."

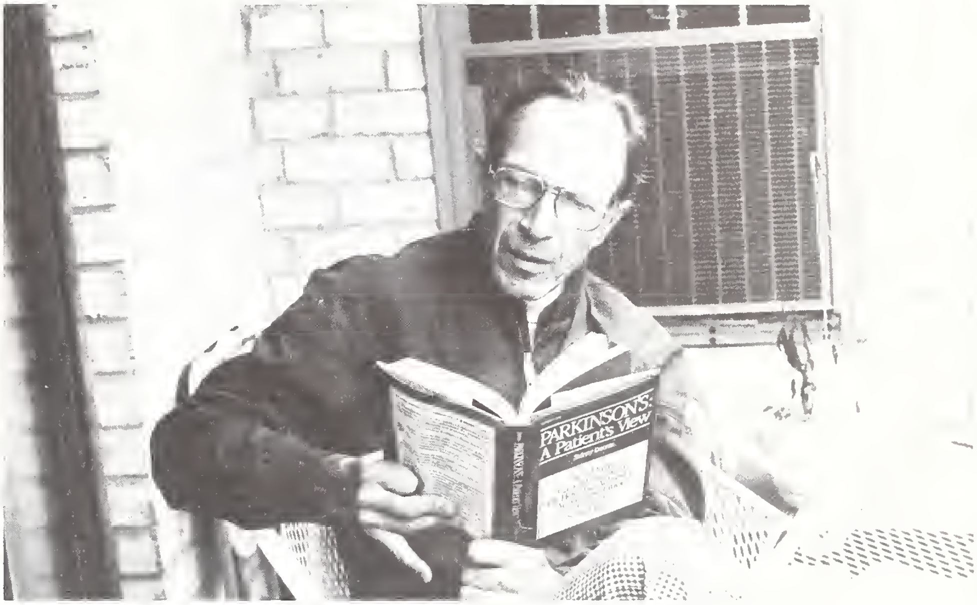
"I feel NIH is one of the most efficient Government agencies," he says. "I have great respect for the objectivity of NIH and for its training of medical personnel: When they do a double-blind study, it's really a double-blind study.

The first double-blind experiment that Dorros participated in at NIH began in 1969. This L-dopa study gave him the most hope for the future. "I went from being a vegetable to having a new lease of life," he remembers. "This was the most important study I participated in. It helped motivate me to get the most out of medication by adjusting my treatment regimen."

In another study, Dorros received L-dopa and carbidopa through an intravenous infusion of the drugs at a constant rate. Results of this intravenous method were compared with other patients' responses to oral doses of the medications. For two days, Dorros fasted and lay flat on his back for 8 hours each day. He managed this ordeal "only with the constant distraction of a pleasant doctor conducting the study and the TLC of the nurses in attendance."

The discomfort was worth it, says Dorros, because when the infusion kept the level of L-dopa in his blood stream at a constant level, the on-off effect—a sudden return of parkinsonian symptoms was eliminated. When Dorros changed his body position or experienced strong emotions, however, extra medication was required to eliminate stiffness or tremor.

"It is, of course, impractical to inject medication into one's veins at a constant rate all day long or to keep variable conditions such as activity or emotions constant," he says in his book. But the study not only convinced Dorros that it is possible for L-dopa to "perfectly control" parkinsonism it also prompted further research by NIH scientists on ways to combat the "on/off" effect.



Sidney Dorros, 56 year old Parkinson's patient reviews his book that he recently wrote to help patients deal with this disorder.

NIH Photo 1771

Although the miracle cure for this effect has not yet been found, Dorros writes: "the finding from the intravenous, infusion experiment that elimination of the 'on/off' effect is theoretically possible gives me hope that the corner will be turned soon."

The experiment also showed Dorros that L-dopa affects him for only one hour and 45 minutes. "That," he says, "has helped me to know how to control my symptoms."

Sidney Dorros continues to work on controlling his symptoms—applying what he has learned through personal observation and through participation in research studies. In his new book, he concludes: "I am still learning to accept my limitations, to utilize my remaining abilities to the utmost, and to achieve the ever-evasive optimum adjustment for medical treatment and living habits. Thus, despite the inevitable problems of life in general and parkinsonism in particular, I am able to enjoy each day as it comes and to anticipate the future with hope." ■

Sidney Dorros' Ten Tips for Coping with Parkinsonism

1. Learn all you can about Parkinsonism and its treatment.
2. Choose a doctor carefully.
3. Become a partner in treatment.
4. Accept and accommodate to your limitations.
5. Make the most of your remaining abilities: Keep active.
6. Take care of your general health.
7. Find purpose in your life.
8. Reach out to help someone: Give and accept love.
9. Have fun.
10. Create your own miracle.

Note: Parkinson's: A Patient's View is available at the NIH Bookstore, Bldg. 10, Rm. B1L101.

Gene Transfer Advance: A Step Closer To Gene Therapy?

rlm

Maureen Gardner

Two separate research groups supported in part by the National Institute of Child Health and Human Development (NICHD) recently achieved a breakthrough in the ongoing effort to understand how genes, the basic units of heredity, are controlled. Their work may ultimately lead to treatment for some of the estimated 2,000 genetic disorders now considered incurable.

Through a carefully timed and delicate microscopic procedure, both teams succeeded in transferring genes to mice from non-mouse sources—a rabbit in one study and a virus in the other. For the first time in animals, transferred "foreign" genes not only worked properly, but also were passed on to offspring.

In both generations of mice, the foreign genes directed certain mouse cells to produce non-mouse proteins, which acted as markers to show when and where the genes worked. This is a valuable system for analyzing the control of genes throughout an animal's development.

According to some scientists, this gene transfer success demonstrates that it may be possible, in time, to treat certain hereditary diseases in man by using healthy human genes to replace ones that are missing or defective. However, many questions about the feasibility and safety of gene therapy will not be answered for years. Meanwhile, the new gene transfer technique may lead to quicker ways to introduce desirable traits into livestock, and will help scientists study one of biology's most mysterious elements, the gene.

Genes, found in the chromosomes of all body cells, are linked sequences of chemical units within strands of DNA. They store our hereditary information and direct the production of substances the body needs to develop and thrive. Estimates of the number of genes in a single human cell range from 100,000 to 2.5 million. If just one malfunctions, genetic disease can result.

Not long ago, it was said that the prospect of gene therapy would be just a fanciful dream until two requirements were met in animal studies. The NICHD-supported studies met both. The first requirement, that the foreign gene must work in its new setting, is very important because scientists hope that in the future a patient with a disease resulting from a missing or non-working gene would be able to receive a healthy, functioning gene that cures the disease.

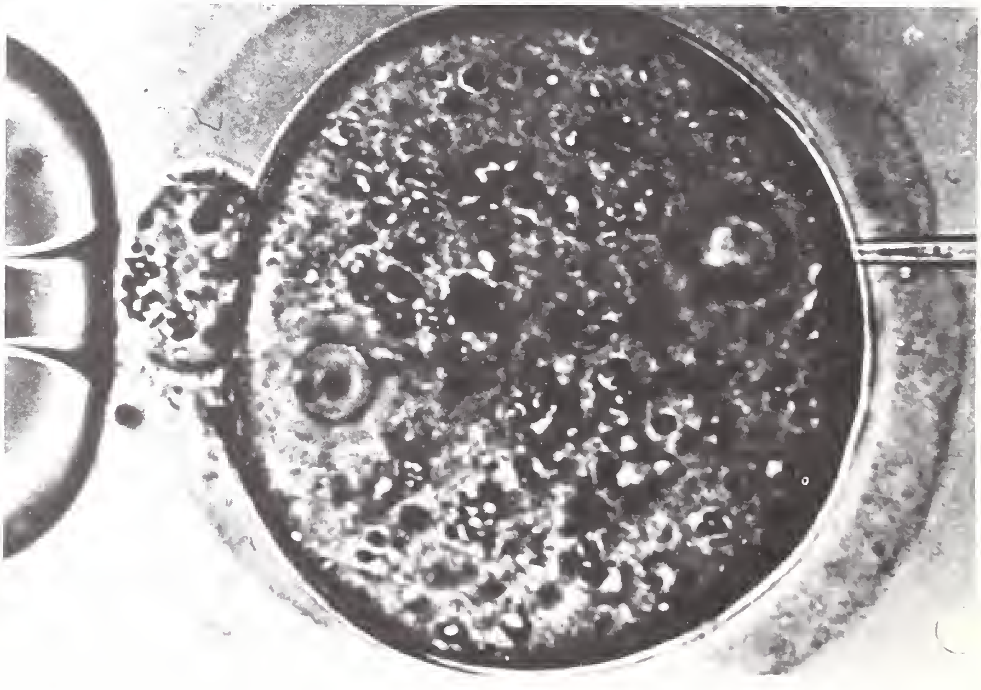
The second requirement, that the working gene must be passed on to the animal's offspring, is equally important. Before human trials can begin, safety must be ensured through years of studies using many generations of animals that transmit foreign working genes to their offspring. In addition, there is hope that someday people with certain genetic diseases will not only be cured themselves, but will pass on only healthy genes to their children.

Earlier gene transfer efforts by other groups were only partially successful. In some studies, transferred genes worked properly—but only in cell cultures, not animals; in other studies, foreign genes were tranferred to animals and passed on to offspring—but did not work there. The recent success of the NICHD-supported teams is thought to be a result of the timing of the gene transfer process.

Working independently, both teams applied the same technique but used different donors for the non-mouse genes. First to report success were scientists at Ohio University led by Dr. Thomas Wagner. This group transferred to mice a rabbit gene that directed the production of beta-globin, a part of the oxygen-carrying molecule in rabbit blood cells. Soon afterwards, a team led by Dr. Ralph Brinster of the University of Pennsylvania and Dr. Richard Palmiter of the University of Washington announced their transfer to mice of a viral gene that directed the synthesis of an easily detectable enzyme called thymidine kinase.

In both studies, fertilized mouse egg cells were removed from mice just after mating. Ready for transfer were thousands of copies of rabbit or viral genes obtained through genetic engineering techniques. The transfer was timed to take place shortly after the egg and sperm had united but before their genetic material combined.

Looking through a microscope, scientists used a hollow glass needle thinner than a human hair to inject as many as 20,000 copies of rabbit or viral genes into the part of the fertilized mouse egg containing the sperm's genetic material. Thus, when the egg accepted the mouse genes from the sperm, it was tricked into taking the foreign genes as well. Many eggs prepared this way were then transferred to uteri of mice who later gave birth, and some of the offspring carried [illegible] functioning non-mouse genes. When these mice matured and were mated, some of their offspring, too, carried the genes.



A hollow glass needle—thinner than a human hair (at right in photo), is used to inject non-mouse genes into a fertilized mouse egg, magnified here 1,200 times. Photo courtesy of the University of Ohio.

NIH Photo 1772

Because the non-mouse genes were incorporated into the developing animal's genetic makeup from the very start, before cell division began, they were passed on to all body cells simultaneously. This is an advantage for scientists wishing to explore how the genes operate in various tissues. It also explains why the genes could be inherited: they were ultimately passed on to the sperm or egg cells of the developing animal.

Researchers do not foresee that this technique will ever be used to inject genetic material into human egg cells. Instead, its success may pave the way for the development of techniques for transferring healthy genes to human tissues involved in genetic disease.

While some scientists feel that it is just a matter of time before gene therapy becomes a reality, others point to some basic questions that still must be answered, such as how a newly inserted gene would stay or work in the appropriate tissue. One point of general agreement is that should gene therapy prove possible, it would most likely be limited to treating hereditary diseases resulting from defects in a single gene. Multigenic diseases, and our physical, mental, and emotional traits (which are influenced by vast arrays of genes) are considered beyond gene therapy's reach for the foreseeable future.

Gene transfer advances such as those accomplished by the NICHD grantees may speed the development of gene therapy techniques; however, ethical criteria would have to be met long before human trials could begin. According to NIH experts Dr. John C. Fletcher and Dr. W. French Anderson, animal studies should show in advance that the probable benefits outweigh the probable risks: there must be reasonable evidence that the new genes will work and will not cause harm.

Other applications of the NICHD grantee's work will most likely come sooner than gene therapy. The livestock industry may make use of the gene transfer techniques to create better breeds more quickly. Another payoff from these studies should be a better understanding of how and why genes switch on and off, guiding countless cells as they differentiate into specialized tissues during development. By tracing foreign genes in experimental animals, scientists hope to begin unravelling the gene regulation mystery. ■

In these studies, mice carrying foreign genes looked just like normal mice, but some of their tissues produced non-mouse proteins that scientists could trace. **NIH Photo 1773**

Brain Hormone Tested as Once-A-Month Contraceptive for Women

Maureen Gardner

A man-made version of a hormone produced in the brain shows potential for becoming a once-a-month birth control drug for women, report scientists at the University of California at San Diego (UCSD). In a preliminary study, injections of the brain hormone induced a condition known to cause infertility in women. If further research brings more success, the hormone could be available in pill form in the coming years.

The hormone tested is a synthetic version of luteinizing hormone-releasing hormone (LHRH), a substance normally produced in the brains of both men and women. Natural LHRH indirectly regulates reproductive processes in both sexes and is essential for fertility.

The synthetic version—called an LHRH-agonist—is about 140 times as powerful as the native form. Scientists have found that this substance can have drastically different effects on the reproductive system, depending on the dosage regimen. While low doses can correct certain types of infertility in women, higher doses can cause infertility in either sex and are therefore under study in the U.S. and abroad as contraceptives for men and women.

The UCSD researchers, supported in part by the National Institute of Child Health and Human Development, devised a new way to apply LHRH as a contraceptive for women. They gave five women an LHRH-agonist injection each day for the first three days of a menstrual cycle. The women's hormone levels were then analyzed daily.

Throughout the treatment cycles, the LHRH-agonist disrupted the complex timing of the release of natural reproductive hormones. Before treatment the volunteers had average 28-day cycles consisting of two 14-day phases, with the cycle midpoint marked by release of an egg from the ovary, or ovulation.

The LHRH-agonist shifted the cycle timing, stretching the pre-ovulatory phase to about 23 days and shortening the second half, called the luteal phase, to about 9 days. Although the cycles lengthened, they remained within normal limits.

For contraceptive purposes, the crucial change was the shortened luteal phase. During a normal 2-week luteal phase, an egg travels from an ovary through a fallopian tube to the uterus, where the lining is prepared to receive that egg, should it be fertilized en route. If the time frame for these events is shortened, the uterine lining is not physically ready when the egg arrives. It is therefore unlikely that a fertilized egg will implant in the uterine wall.

This shift in cycle timing occurs naturally in some women: it is an uncommon condition, a cause of infertility called luteal phase defect. The UCSD scientists, led by Dr. Samuel Yen, are the first to use a drug to induce luteal phase defects in humans. Further study is needed to determine whether the LHRH-agonist will work consistently with extended use.

Dr. Yen points out that UCSD findings are particularly exciting because they show an easy reference point for when to take the LHRH-agonist—the start of the menstrual period. Thus, if the synthetic hormone proves to be an effective birth control drug, it will offer the convenience of a once-a-month dose with a natural reminder to take it.

Before LHRH-agonists can be marketed as contraceptives, they must undergo large-scale clinical trials to evalute their effectiveness and safety. In studies to date, they appear to confine their actions to the reproductive system alone. Because of this, they are expected to be safer than present hormone-based contraceptives containing steroids, a type of hormone that can cause side effects in many systems of the body.

Dr. Yen predicts that LHRH-agonists will be ready for clinical trials in 2 to 3 years. "The whole world is waiting for a better birth control pill," he states, "but it is not going to come easily." Dr. Yen's group is studying ways to produce LHRH-agonists in more convenient forms than injections now used, such as capsules that can be absorbed under the tongue. ■

Locating Opiate Receptors Gives Clues About Brain Functions and Disorders

Marilyn Sargent

Because neuroscientists have now found that opiate receptors are located throughout the brain, they believe that internally manufactured opiate peptides may have a role in normal brain function that goes beyond killing pain.

"Given the widespread, yet intricate and discrete, localization of their receptors, endogenous opiates may be a basic neurotransmitter class, modulating neural activity and brain function in ways we do not yet understand," said Dr. Miles Herkenham, neuroanatomist with the NIMH Laboratory of Neurophysiology.

Research showing that opiates have an effect on sensory perception, eye movements, locomotor activity, repiration, and heart rate indicates that opiate receptors are present in the parts of the brain that mediate those functions, Herkenham explained. "Opiates are analgesics because they interact with opiate receptors located in the parts of the brain that mediate pain and not because the opiates themselves have effects such as those produced by traditional anesthetic agents."

A surprising finding, he continued, is the extreme differences in receptor distribution among species. "For example, rat hypothalamus has very few opiate receptors whereas in rhesus monkey brain the highest density of opiate receptors is located is some hypothalamic nuclei. Humans have extremely high densities of opiate receptors in the cerebral cortex." These findings strongly suggest that opiates play different roles in different species, said the investigator.

Major advances in the localization of opiate receptors in the brain have been made possible by a technique developed by Herkenham and Dr. Candace Pert, NIMH Biological Psychiatry Branch. Referred to by Herkenham as "a new way for understanding drug and neurotransmitter action," the technique permits postmortem marking of drug and transmitter binding sites in brain tissue sections in which biological activity is still intact.

Using autoradiography, pictures are made that show where radioactively labeled drugs bind to their receptors. When seeking locations of opiate receptors, for instance, radioactively labeled morphine can be used. For locating a dopamine receptor, a radioactively labeled neuroleptic is used.

"An exciting feature," said Herkenham, "is that we can map receptor distributions in the brain after death, which permits us to study human brain tissue."

The scientist and his colleagues already have begun to examine opiate and dopamine receptor distributions in the brains of deceased schizophrenics and individuals who died with neurologic disorders such as Parkinson's disease.

Based on knowledge that antipsychotic neuroleptic and anti-Parkinsonian drugs work via their interaction with dopamine receptors, biological theories hold that the disorders treated by these drugs involve some defect in the dopamine receptor system, Herkenham explained. Possibly there are too many or too few receptors, or they may be improperly regulated.

"By comparing the brains from these patients with brains of those who died with no indication of neural dysfunction, we may find differences in receptor locations or determine if there are quantitative or regulatory differences."

Tracking dopamine receptors with the idea that they are involved in mental illness is particularly difficult, observed Herkenham, because neuroleptic drugs bind not only to the dopamine receptor but to other sites in the brain. Nevertheless, it is instructive to note where they do bind."

The challenge of locating opiate and neuroleptic/dopamine receptors is part of a larger goal described by Herkenham as "finding the structural correlates of the dynamic brain." For example, he explained, receptors, which are cell membrane components, are the structural sites where dynamic intercellular communication takes place.

"Our long-term plans include mapping receptors in the cerebral cortex." A complete map awaits the discovery, by other scientists, of the significant neurotransmitters in the major motor and sensory pathways of the cortex, he said.

"By mapping receptor distribution in the brain, we are laying the foundation for learning which pharmacological agents function in which parts of the brain. This information will, in turn, provide insights into the complex brain mechanisms that produce normal functioning as well as the malfunctioning that results in mental and neurological disorders." ■

Chickenpox Vaccine Studied for Protection of Leukemic Children

Joan B. Hartman

Five hundred leukemic children will participate in a multi-center clinical study to see if a live attenuated varicella vaccine will protect them from potentially fatal chickenpox. The study, to be conducted at 27 medical facilities across the country and in Canada, is supported by the National Institute of Allergy and Infectious Diseases (NIAID).

Chickenpox, caused by the varicella-zoster virus, is a mild disease for normal children. The latent vrius later can cause the disease known as shingles (zoster), a painful and potentially debilitating condition that usually occurs in the middle or later years. It occasionally is seen in childhood following chickenpox in immunosuppressed patients. Because chickenpox usually is harmless, although the latent virus can cause subsequent disease, there has been little interest in developing a varicella vaccine for normal children.

But for children with suppressed immune systems, such as leukemic children undergoing chemotherapy, varicella can be a severe, complicated and sometimes fatal disease. In one study of 77 children with cancer who developed chickenpox, seven percent died of the virus infection. Most of the children had leukemia.

Children enrolled in the current study will be under 15 years of age. To be eligible they will have been in remission from acute lymphocytic leukemia (ALL) for at least one year. They will have been off chemotherapy for from two to six months or at a stage of chemotherapy that can be stopped safely for at least two weeks without jeopardizing that remission. None will have had radiation therapy for at least six months prior to vaccination. The children must never have had chickenpox, and they must have no antibodies to the varicella-zoster in their blood.

In order to test the efficacy of the vaccine, only children who might be exposed to chickenpox through a household contact will be eligible for the study. Each child enrolled must have a sibling or parent who also has not had chickenpox and who also is negative for varicella-zoster antibodies.

Statistical averages indicate that of the 500 children vaccinated, approximately 20 will have household exposure to chickenpox in the two years following vaccination. Without vaccination, 90 percent of [illegible]se 20, or 18 children, would be expected to develop chickenpox after exposure. Investigators believe it will be possible to determine how effective the vaccine is by comparing this probability of infection with the number of children who actually develop chickenpox in the two years following vaccination.

In addition, investigators intend to follow for as long as possible both vaccinated children and an equal number of control children (leukemic children who have had natural varicella but no vaccination) to determine whether children in either group develop shingles and, if so, if the disease occurs more frequently in one group than in the other.

The varicella vaccine to be used, Takshaski Oda strain, was developed in Japan and has been used effectively in studies there to protect high risk children. The purpose of the NIAID study is to confirm and extend the results of the Japanese study.

Principal investigator and coordinator of the study is Anne A. Gershon, M.D., associate professor of pediatrics, New York University School of Medicine. Of the institutions proposed for inclusion in the study, 20 have submitted documentation necessary for participation in a federally funded study. Others are expected to complete this process soon. The initial 20 and their institutional co-investigators are:

New York University Medical Center, Anne A. Gershon, M.D.: St. Jude's Children's Research Hospital, Walter Hughes, M.D.; Nassau County (NY) Medical Center, Avron Ross, M.D.; Children's Hospital, Sidney Farber Cancer Center (Boston), John Modlin, M.D.; Memorial Sloan Kettering Institute (NYC), Arthur Brown, M.D.; Stanford University Medical Center, Ann Arvin, M.D.; North Shore University Hospital (Long Island), Jospeh Kochen, M.D.; Columbia Presbyterian Hospital (NYC), Sergio Piomelli, M.D., Geisinger Medical Center (Danville, PA), Narayan Shah, M.D.; Children's Memorial Hospital (Chicago), Helen Maurer, M.D.; Emory University Medical Center, Abdel Ragab, M.D.; St. Jude's Midwest Affiliate (Peoria, IL), Joyce Constine Wallenberg, M.D.; University of Oklahoma Medical Center, Melvin Marks, M.D.; Johns Hopkins University Medical Center, Brigid Leventhal, M.D.; Duke University Medical Center, Mary V. Moggio, M.D.; University of California at Los Angeles, Stephen Feig, M.D.; Medical College of Virginia, Harold Mauer, M.D.; University of Texas Health Sciences Center at Dallas, George R. Buchanan, M.D.; and Mt. Sinai Medical Center (NYC), Michael Weiner, M.D. ■

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Dental Survey Finds Decline in Tooth Decay in School-Aged Children

Prepared by the National Institute of Dental Research Information Office

There has been a notable improvement in the dental health of U.S. school children, according to recently released results of a 1979-1980 national survey conducted by the National Caries Program of the National Institute of Dental Research (NIDR).

Tooth decay is still the leading chronic disease of childhood. The survey reported a one-third reduction in dental caries (tooth decay), which represents a dramatic savings in the annual expenditure for dental care, which, in 1980, was reported to be approximately $16 billion.

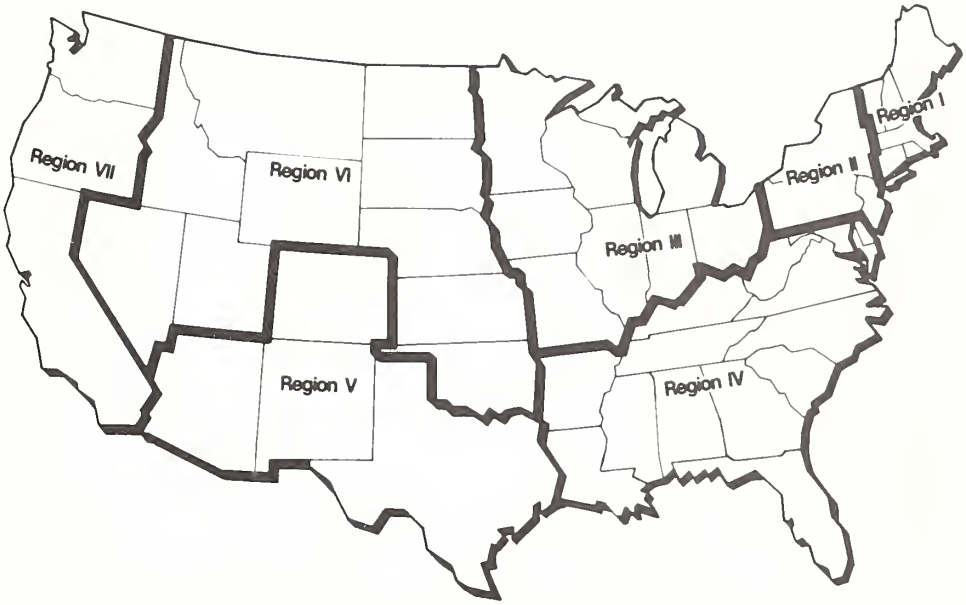
Compared with a study conducted in 1971-1973 by the National Center for Health Statistics, the prevalence of dental caries among children between the ages of 5-17 has decreased from 7.1 to 4.8 decayed, missing and filled permanent tooth surfaces per child during the last eight years.

The survey included only children between the ages of 5-17 enrolled in public or private schools. A probability sample of approximately 40,000 children in seven broad geographic areas was drawn to represent the 48 million school children in the United States.

The results of the survey revealed that almost 17 million children, aged 5-17, were caries free—no decayed, missing or filled permanent teeth. The percentage of caries-free children for selected ages was 95% of all five year olds, 51% of nine year olds, 27% of twelve year olds and 12% of sixteen year olds. The prevalence of caries in 1980 was lower for every age group studied.

Although the National Caries Program cannot offer any single definitive reason for the decrease in caries prevalence, it is believed that a number of factors have contributed to this reduction. One of the most important factors is community water fluoridation, which was adopted in many U.S. cities within the last 30 years. In addition, there has been a tremendous increase in the use of fluoride toothpastes. Professional applications of fluoride in dental offices appears to more widely used today.

Also, during the past 10 years, the continued growth of school-based programs of weekly mouthrinsing with solutions of fluoride tablets have helped to decrease the prevalence of dental caries. Over 12 million school children are presently taking part in these caries prevention programs, as a result of the promotional efforts of the National Caries Program. ■



The 1979-80 National Dental Caries Prevalence Survey sampled seven geographic regions in the United States. The data are based on the dental examinations of 37,956 school children.

NIH Photo 1774

Scientists Develop New System for Studying DNA Synthesis

Doris Parker

A long sought system recently developed in the Stanford University laboratory of Nobel Prize winner Arthur Kornberg should significantly ease the way for researchers studying cell growth and cancer.

The newly discovered system, which initiates bacterial DNA replication—the beginning of cell division and growth—in the test tube, can now be manipulated in a controlled environment by scientists. As a result, crucial questions, including those concerning the biochemistry of uncontrolled growth, can be asked with greater chance for useful answers.

Although Dr. Kornberg and his coworkers, Robert Fuller and Jon Kaguni, feel that the new system can eventually make possible an understanding of many biological puzzles, including mutation, embryonic development and carcinogenesis, they caution that the system is only a beginning—that it is a tool for further research.

The biochemistry of many basic operations performed by DNA molecules is essentially identical in bacteria, plants, animals and humans. Thus Dr. Kornberg feels sure that an understanding of DNA replication in bacteria will give reliable insights into replication in higher organisms. "There is a universal use of basic biochemical patterns throughout nature that warrants our confidence that regulation of these processes, when understood in one system, will become far easier to understand in all systems," he said.

This plasmid is a short circular piece of DNA that contains the "origin", the segment of DNA from the **E. coli** chromosome where DNA replication always begins. (This photo was taken by a electron micrograph).

Kornberg and his colleagues have determined that DNA replication proceeds simultaneously in both directions along the DNA chain from the origin of synthesis.

NIH Photo 1776

Dr. Arthur Kornberg (center) discusses DNA replication with his coworkers, Jon Kaguni (who photographed the electron micrograph on opposite page) and Robert Fuller (right).

NIH Photo 1775

Dr. Kornberg has worked on the biochemistry of DNA throughout his research career. The activities directed by this molecule in the course of carrying out its two major functions—the building of proteins required for all life processes and its own replication—have proved to be amazingly complex. Indeed, DNA's operations have proved to be far more complicated than scientists dreamed possible in 1953 when James Watson and Francis Crick deduced the molecule's simple, elegant double-helix structure.

In 1959, Dr. Kornberg won the Nobel Prize for his work in synthesizing DNA in the laboratory. In 1967, he reported that he had made DNA that showed biological activity, but developing the system essential for replication was still years away.

"We sought this system for many ye without success," Dr. Kornberg recall "Many other laboratories around the world were also working very hard on this problem."

After years of work, he and his coworkers located the segment of DNA in *E. coli* (a much-studied bacterium) that contains the region responsible for initiating replication. Since this segment worked only inside an intact cell, they knew that many factors in the cell must be necessary to switch on the process.

The task was to extract these factors, but first they needed to obtain much more of the segment—called the origin. They turned to recombinant DNA technology, splicing origin segments into plasmids—tiny rings of extrachromosomal DNA that occur in most bacterial cells. When the hybrid plasmids were taken up by the *E. coli* cells, as many as 100 copies of the segment could be made and harvested from each cell.

The next step was getting the segment to work in the test tube. The trick was isolating just those enzymes necessary to "throw the switch."

They found that neither adding mixtures of purified enzymes known to be involved in DNA synthesis nor adding fluid containing the entire cell contents could initiate replication. But for some reason, ammonium sulfate—added to the cell contents in just the right amount—triggered the burst of replication they were looking for. It is not yet known whether the ammonium sulfate caused concentration of the ingredients necessary for initiation or caused some substance inhibiting initiation to be inactivated. These are some of the questions now to be answered.

Dr. Kornberg's group will now sort out the components of the system and begin to analyze very precisely what substances are required for it to work. "Why it took so long to find this DNA synthesis initiation system will be answered, in part, by finding out precisely what's in the system that is exacting and irreplaceable," Kornberg said.

The pieces of the DNA puzzle are beginning to come together, and the possibilities for further advances are tantalizing. However, as Lewis Thomas said in his book *The Medusa and the Snail*, "If it is really good science, it is impossible to predict how it is going to turn out...we are making a beginning and there ought to be some satisfaction, even exhilaration, in that."

This research was supported by grants from the National Institute of General Medical Sciences and the National Science Foundation, as well as by fellowships from the American Cancer Society and the Damon Runyon—Walter Winchell Cancer Fund. ■

Exercising Monkeys on Fatty Diets Protected from Heart Disease

Laura Winston (student intern for the News Branch)

A recent study provides new evidence that moderate exercise may protect against coronary atherosclerosis, the number one killer of middle-aged men in the United States.

The study, funded by the National Heart, Lung and Blood Institute at the National Institutes of Health, suggests regular exercise may slow or prevent development of atherosclerotic heart disease in monkeys. This finding provides added evidence that exercise may also be protective in man.

Coronary atherosclerosis remains the 20th century epidemic of the western world. Although a reduction in the number of deaths has occurred, the value of exercise or any other preventive measures has not been definitely proven. Factors such as diet and lifestyle as well as amount of exercise, may influence a person's chances of developing the disease.

In this study, Dr. Dieter Kramsch and associates from Boston University Medical Center, observed that exercise favorably affected the coronary arteries of monkeys fed "fatty diets."

Earlier studies using birds and lower mammals have produced conflicting evidence that exercise lessened experimentally-produced atherosclerosis.

In coronary atherosclerosis, yellowish plaques made up of fatty material clog the inner lining of the coronary arteries. Blood flowing to the heart cannot pass through the clogged arteries easily and insufficient oxygen may trigger a heart attack.

The researchers fed certain monkeys a fatty diet designed to produce changes in their coronary arteries similar to those seen in atherosclerosis. Twenty-seven monkeys were divided into three groups, depending on the kind of diet and amount of exercise they received. Two groups were considered sedentary and could only exercise within the confines of their cage.

The third (exercise) group was conditioned on a treadmill until they could sustain speeds of 2 to 3.5 kilometers per hour (about 1¼ to 2 mph). According to the researchers, this is comparable to jogging in humans. The monkeys ran for one hour three times a week for three and a half years.

One group of sedentary monkeys ate a normal diet of Purina monkey chow and banana mash, while the other sedentary group and the exercise group ate the normal diet with cholesterol and butter added.

The investigators found that the sedentary monkeys on the fat diet, developed signs of coronary atherosclerosis. With various tests they noted that the inner lining of the coronary arteries had thickened and narrowed.

In contrast, the exercising monkeys had much less thickening of the inner lining of their coronary arteries. Like an elastic tube, the arteries actually widened so blood was easily able to flow to the heart.

In addition, the left ventricle of the heart in these monkeys increased in size and functioned at a slower rate, allowing their bodies to pump blood more efficiently as well.

The scientists believe that another way the monkeys may be protected against atherosclerosis is by increased levels of high density lipoproteins in the blood.

A favorable ratio of high density lipoproteins to low density and very low density lipoproteins helps monkeys and humans digest the fats in the body. An unfavorable ratio causes the fatty material to be deposited in the coronary arteries.

The study showed that monkeys in the exercise group had small but significant increases in high density lipoproteins in their blood and large decreases in low density and very low density lipoproteins compared with the sedentary monkeys on the fat diet.

Higher levels of high density lipoproteins in the blood are associated with lower risk of coronary artery disease, according to Dr. Edwin Gangloff, program administrator at the National Heart, Lung, and Blood Institute.

However, he said the results of this study only suggest a link between exercise and protection against coronary atherosclerosis.

Gangloff said it is difficult to predict the effect of exercise on prevention of disease in humans from experiments with monkeys.

More research must be done on animals and humans before definite statements can be made about the relationship between exercise and protection against atherosclerosis.

A report of the study appeared in the December 17, 1981 issue of the *New England Journal of Medicine*. ■

National Brain Tissue Banks: A Service for Neurological Research

Bill Cole

The human brain is by far the most complex organ of the body. It is also the most difficult organ for medical investigators to study: inaccessible to them during a patients's life, too often unavailable to them after a patient's death. Yet an adequate supply of both normal and diseased brain tissue is essential for the research that will eventually enable scientists to conquer neurological disease.

When removed from the body shortly after death and preserved, the brain can reveal many of its innermost secrets. With the electron microscope and other sophisticated new technologies, nerve cells can be minutely observed and tissue examined for the lesions or damage wrought by brain disease. Sometimes, as in Parkinson's disease, such studies help scientists identify the abnormality responsible for the damage, and give them clues for controlling or preventing the disorder.

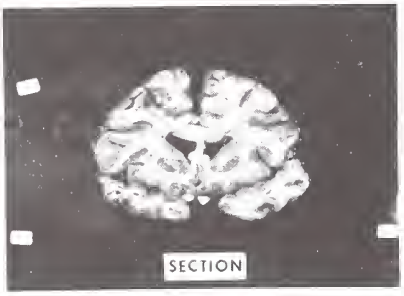
Because of such advances, the future holds real promise for a better life for patients with neurological disease. "The next ten years should allow us to reap the benefits of the revolutionary advances in neuroscience," says Dr. Murray Goldstein, Acting Director of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS), the major federal agency responsible for research on the brain and central nervous system.

But progress in understanding and preventing nervous system disorders depends upon the generosity of patients, their families, and their friends in donating brain tissue as a legacy and a hope for the future.

Before brain tissue can be used in a

PROCESSING THE TISSUE

CUTTING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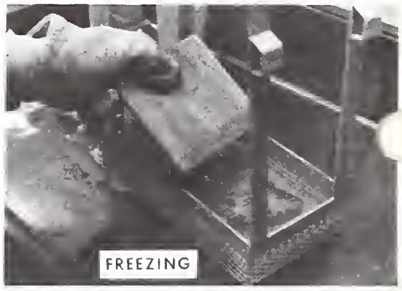
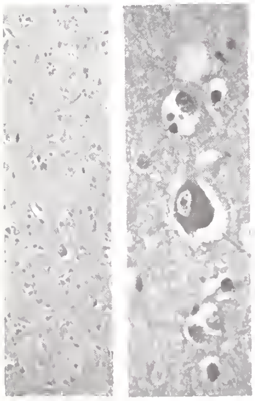
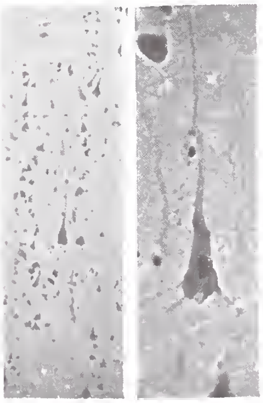

FREEZING

PHOTOMICROGRAPHS

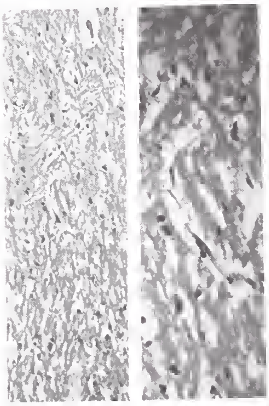
NOT FROZEN

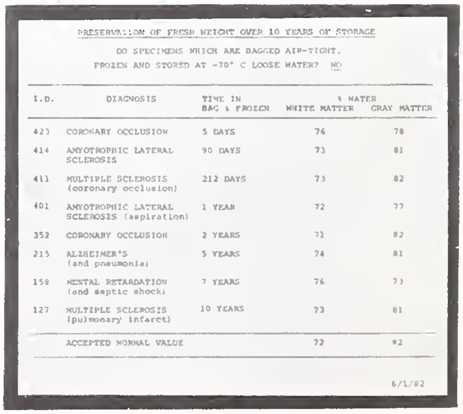


QUICK FROZEN



SLOW FROZEN





PRESERVATION OF FRESH WEIGHT OVER 10 YEARS OF STORAGE

DO SPECIMENS WHICH ARE BAGGED AIR-TIGHT,
FROZEN AND STORED AT -70° C LOOSE WATER? NO

I.D.	DIAGNOSIS	TIME IN BAG & FROZEN	% WATER	
			WHITE MATTER	GRAY MATTER
423	CORONARY OCCLUSION	5 DAYS	76	78
414	AMYOTROPHIC LATERAL SCLEROSIS	90 DAYS	73	81
411	MULTIPLE SCLEROSIS (coronary occlusion)	212 DAYS	73	82
401	AMYOTROPHIC LATERAL SCLEROSIS (aspiration)	1 YEAR	72	77
352	CORONARY OCCLUSION	2 YEARS	71	82
215	ALZHEIMER'S (and pneumonia)	5 YEARS	74	81
158	MENTAL RETARDATION (and septic shock)	7 YEARS	76	73
127	MULTIPLE SCLEROSIS (pulmonary infarct)	10 YEARS	73	81
	ACCEPTED NORMAL VALUE		72	82

6/1/82

After an individual's death, the diseased or healthy brain is removed and studied to reveal the many secrets involved in neurological disorders.

These tissues are cut, sectioned, bagged and often frozen for storage to be used later in further studies.

These brain tissues help researchers gain knowledge in neurological disorders such as alzheimer's amyotrophic lateral sclerosis, coronary occulusions and multiple sclerosis.

NIH Photo 1777

laboratory for scientific research, it must be collected from donors, quick-frozen, and cryopreserved under conditions that will protect the fragile tissue. Then a distribution system must be in place to deliver the tissue to investigators. A few large medical institutions have their own "brain banks," as they are called, but most research centers do not have the resources, facilities or specialized knowledge for such an operation.

To satisfy the growing demand for brain tissue, the NINCDS, together with other federal agencies, has helped to establish two national brain tissue resource centers, one in Los Angeles, the other near Boston.

These national brain tissue resource centers supply investigators outside their own institution with tissue. In addition to this vital service, investigators associated with the brain banks conduct research of their own. And, if a donor or family wishes, the brain bank will make all arrangements for donations.

In Los Angeles, the NINCDS-supported National Neurological Research Bank is located at the Veterans Administration Wadsworth Medical Center, under the direction of Dr. Wallace W. Tourtellotte. Currently, the bank accepts tissue taken at autopsy from patients with the following disorders, multiple sclerosis, Huntington's disease, epilepsy, Parkinson's disease, amyotrophic lateral sclerosis, hereditary ataxia, dementia, Alzheimer's disease, senile dementia, subacute sclerosing panencephalitis, Tourette syndrome, mental retardation, schizophrenia, manic depression, psychosis, suicide, and drug or alcohol abuse. Tissue from elderly donors is also needed.

The second NINCDS-supported brain bank, the Brain Tissue Resource Center, is located just outside Boston at McLean Hospital in Belmont. It is under the direction of Dr. Edward D. Bird, and deals primarily with tissue from patients with Huntington's disease, Parkinson's disease, Alzheimer's disease, schizophrenia, Tourette syndrome, and dystonia.

Both brain banks plan to expand their work to include more disorders, and both will respond promptly to inquiries about brain tissue donation by patients or families afflicted with *any* incurable disorder of the central nervous system.

Both brain banks also need donations of normal brain tissue. In many scientific studies, each sample of diseased tissue must be matched with a sample from a normal brain—one without neurological disease. Scientists can then compare the two biochemically, structurally, and otherwise, to determine how they differ and how tissue changes in disease.

Normal tissue can be donated by anyone. Preferably, normal tissue should come from someone of the same age, sex, race, and socioeconomic background as the donor of the diseased tissue with which it is matched. When there is a large supply of both diseased and normal tissue, investigators have no difficulty finding a suitable match.

Normal tissue is usually the most difficult to obtain. One chief source is donations from healthy family members of patients with neurological disease. But even people who do not have a personal involvement in neurological disease may be willing to donate brain tissue once they become aware of the great value of such a gift.

So far as we know, animals are not susceptible to Parkinson's disease, multiple sclerosis, Alzheimer's disease, Huntington's disease, or many other disorders that afflict the human brain and central nervous system. So laboratory models can only imperfectly substitute as systems for the study of these devastating illnesses. Much of the research needed to prevent, control, and eradicate these disorders must be conducted on human brain tissue.

"About 20 years ago, it became clear that if we were going to understand brain disorders we would have to study the actual brain tissue after death," says Dr. Bird, a physician and neurochemist who is associate professor of neuropathology at Harvard Medical School.

One success story Dr. Bird enjoys recounting is the breakthrough that occurred through the study of brain tissue from Parkinson's disease patients. On studying chemical substances in these brains, scientists found a deficiency of dopamine, a chemical messenger that transmits signals along nerve cells and that is essential for proper body function. A substitute for dopamine—the drug L-dopa—was eventually developed, helping to relieve symptoms of trembling and muscle stiffness in many Parkinson's disease patients.

Encouraged by this success, more scientists started collecting and studying brain tissue. Other chemicals involved in the transmission of impulses and messages along nerve cells and fibers were identified and associated with specific neurological disorders.

For example, in studying the abnormal tangles of nerve fibers found in the brains of patients with Alzheimer's disease (the most common cause of intellectual impairment in older persons), scientists found a sharp reduction—as much as 90 percent—in the brain enzyme choline acetyltransferase. This enzyme is involved in the passage of brain signals. If further studies reveal that this chemical deficiency is responsible for Alzheimer's disease symptoms, scientists may be able to develop new drugs to treat Alzheimer's disease in much the same way as L-dopa is used to treat Parkinson's disease.

In one of the most dramatic advances made possible by donated brain tissue, NINCDS investigators discovered that certain neurological disorders are caused by "slow" viruses that lie dormant in nervous tissue for years before they are activated. Study of the brain tissue of patients who suffered from subacute sclerosing panencephalitis (SSPE), a form of inflammation of the brain, showed that the disorder was caused by a type of measles that may have been contracted in childhood long before the appearance of SSPE symptoms.

These findings suggest that slow viruses or some other infectious agent may be a cause of demyelinating disorders such as multiple sclerosis, a progressively disabling neurological disorder that primarily affects young adults. Many investigators believe that there is also an abnormal immune response factor in multiple sclerosis, and that the body's immune defense system becomes deranged and attacks the body's own tissue. These theories, which hold the hope of more effective treatment and eventual prevention of disease, can only be proved through the intensive study of human brain tissue.

Symptoms of some behavioral disorders are similar to those of disorders of the central nervous system, and patients may be incorrectly diagnosed. But brain tissue changes observed in postmortem examination can correct the diagnosis. A more certain diagnosis is especially important in families with hereditary neurological diseases, so descendants can make informed choices about marrying and having children.

Dr. Bird tells of the time that he and his associates examined brain tissue from patients diagnosed as having schizophrenia and found that they actually had had Huntington's disease—an inherited neurological disorder that ravages both the minds and bodies of its victims. As each child of a parent with Huntington's disease has a 50 percent chance of inheriting the disorder, it was vital to inform surviving family members of the presence of the disease in the family, and of the risk

continued on next page

of passing the defective gene on to succeeding generations.

But sometimes the news is good. Suppose in this case the condition had been incorrectly diagnosed as Huntington's disease or some other severe inherited neurological disorder. Postmortem examination of the brain could show that the diagnosis was wrong, and that no hereditary factor was involved. With such knowledge surviving family members would no longer have to fear falling victim to the disease themselves to having an affected child.

Dr. Tourtellotte, who in addition to directing the Los Angeles brain bank is professor and vice-chairman of neurology at the University of California in Los Angeles, has been collecting brain tissue from patients for 20 years. Like Dr. Bird, he believes that the best way to get brain tissue donations is to appeal directly to patients and their relatives.

"These are the people who have the strongest desire to support brain research," Dr. Tourtellotte says. "There is no substitute for brain tissue donations from patients and healthy individuals. There can be no more sincere or valuable gift to mankind."

Dr. Tourtellotte points out that a single donation can serve many purposes. "Only a very small amount of tissue is needed for chemical analysis," he says, "so a single brain can provide material for more than 100 studies. It's a splendid contribution."

Donated brain tissue is collected only after a person's death. For people wishing to make donations, Drs. Tourtellotte and Bird stress the following:

—Arrange for donations as soon as you are told by your doctor that you have a serious neurological disease. You can also donate brain tissue if you do not have such a disease but want to help support brain research. Chemical changes occur rapidly after death, so the brain must be removed as soon as possible, preferably within 24 hours. For your donation to be of maximum value to science, all arrangements must be made well in advance.

—Anyone 18 years of age or older and of sound mind may become a brain donor simply by signing an organ donation card. If you are under age 18, a parent or a legal guardian must consent to your being a donor. If the donor cannot sign the card but has given no indication that such a gift should not be made, the next of kin may give consent. The next of kin in order of survival means (1) the spouse, (2) an adult son or daughter, (3) either parent, (4) an adult sister or brother, (5) a guardian, or (6) any person authorized to make arrangements for the deceased. Permission for the autopsy and donation of brain tissue must be obtained again from the next of kin after the patient's death, but the wishes of the deceased will almost always be respected if they are well known in advance.

—Donor forms and organ donation cards can be obtained from the Los Angeles and Boston brain banks (see addresses below). Carry the card at all times. Your family and physician should be informed of your wishes, to ensure their cooperation and avoid delays in carrying out the autopsy. Donors of brain tissue may also participate in other organ donor programs, such as those involving the eyes or kidneys.

—If, after signing a donor card, you change your mind about donating, you can leave the program simply by tearing up the card. If you do so, be sure to tell your family and physician.

—Brain donations do not affect funeral and burial ceremonies in any way. An autopsy leaves no scars visible in a normal funeral.

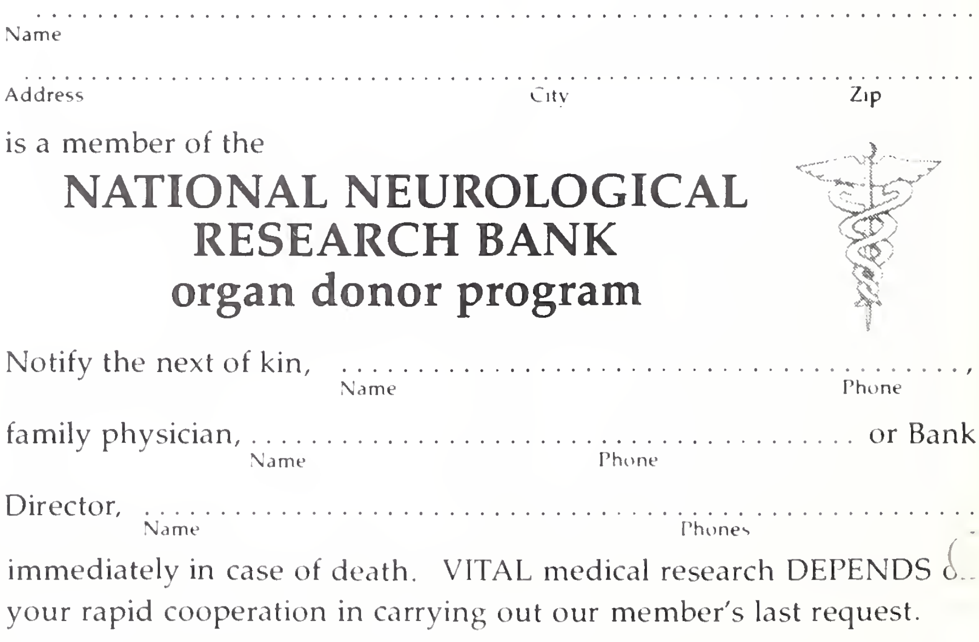
—In addition to providing priceless research material, the autopsy may help the surviving family by supplying a definitive cause of death. Or the autopsy may reveal the presence of previously unsuspected infectious or inherited disease, in which case the family can be informed and counseled.

—Most large hospital provide autopsies without charge to the family. But if death occurs outside a large hospital, a pathologist must be brought in to perform the autopsy and a fee is sometimes charged. In selected cases, the NINCDS-supported brain banks may be able to provide some funds for the autopsy—but only if arrangements for such payment are made in advance. After the autopsy, the brain banks assume all costs in handling donated tissue. But the brain banks cannot pay funeral costs, nor can they pay donors or families for tissue.

—Besides eventual donation of brain tissue, patients with neurological disorders can make valuable contributions to research while they live. They can undergo periodic medical examinations, including the drawing of small amounts of blood and spinal fluid to help scientists trace the course of their disease.

Information about donating brain tissue can also be obtained from many of the voluntary health agencies working to combat neurological disorders and to help patients and their families. These agencies work in close cooperation with NINCDS and the national brain tissue banks. Contact the local chapter of the voluntary health agency (or agencies) dealing with the disorder in which you are interested. If there is no chapter in your area, contact the national headquarters.

There are hundreds of neurological di orders. Because of limited resources staff, the NINCDS-supported brain banks can deal only with a few disorders at a time. The voluntary health agencies can tell you of other smaller brain banks specializing in specific conditions, where donations are urgently needed and gratefully accepted.



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Name

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Address City Zip

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NATIONAL NEUROLOGICAL
RESEARCH BANK
organ donor program

Notify the next of kin, ...,
Name Phone

family physician, ... or Bank
Name Phone

Director, ...
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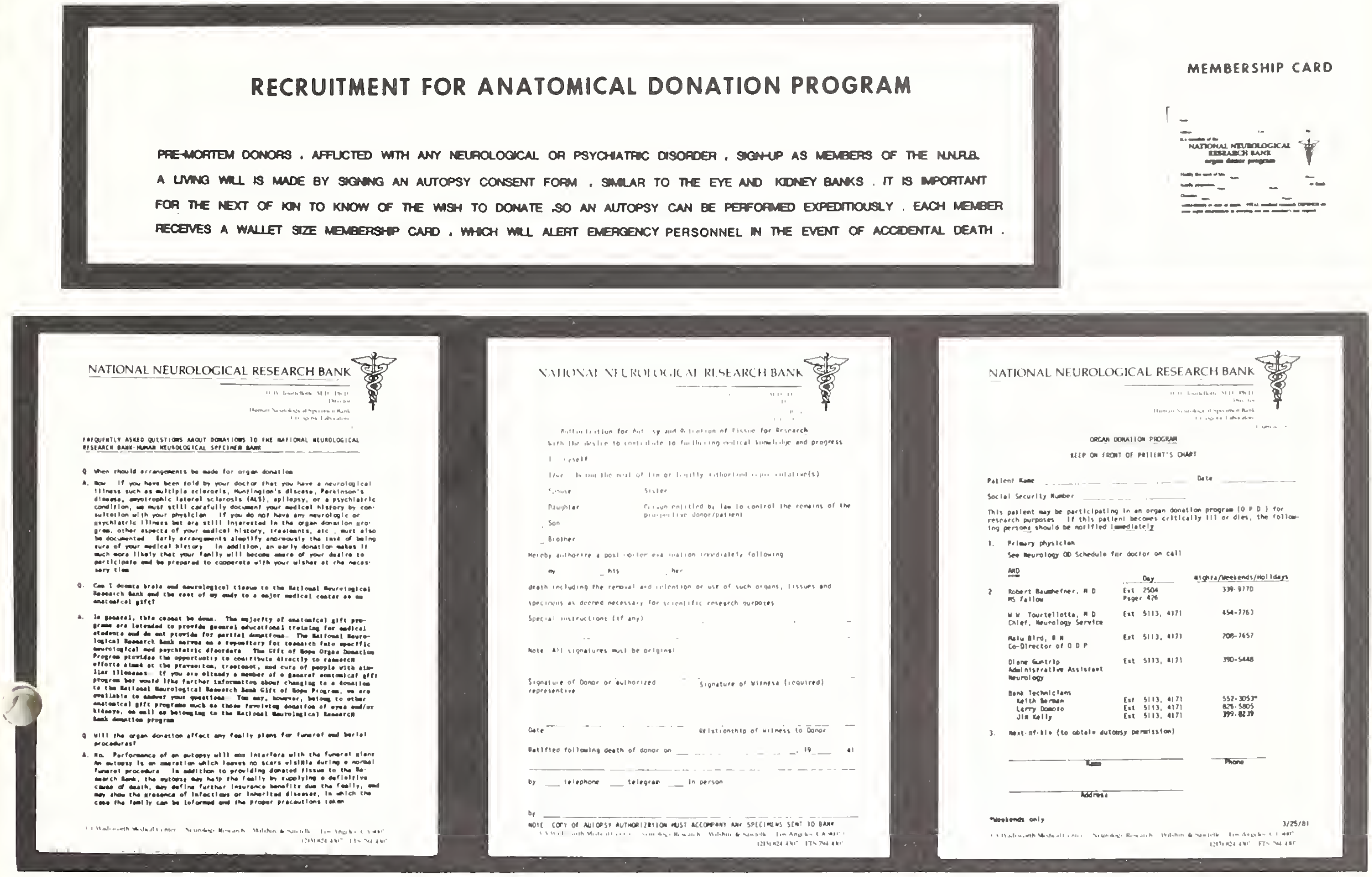
immediately in case of death. VITAL medical research DEPENDS o
your rapid cooperation in carrying out our member's last request.

The donor card—key to a priceless gift.

NIH Photo 1778



RECRUITMENT FOR ANATOMICAL DONATION PROGRAM

PRE-MORTEM DONORS , AFFLICTED WITH ANY NEUROLOGICAL OR PSYCHIATRIC DISORDER , SIGN-UP AS MEMBERS OF THE N.N.R.B. A LIVING WILL IS MADE BY SIGNING AN AUTOPSY CONSENT FORM , SIMILAR TO THE EYE AND KIDNEY BANKS . IT IS IMPORTANT FOR THE NEXT OF KIN TO KNOW OF THE WISH TO DONATE ,SO AN AUTOPSY CAN BE PERFORMED EXPEDITIOUSLY . EACH MEMBER RECEIVES A WALLET SIZE MEMBERSHIP CARD , WHICH WILL ALERT EMERGENCY PERSONNEL IN THE EVENT OF ACCIDENTAL DEATH .

MEMBERSHIP CARD

NATIONAL NEUROLOGICAL RESEARCH BANK
organ donor program

NATIONAL NEUROLOGICAL RESEARCH BANK

FREQUENTLY ASKED QUESTIONS ABOUT DONATIONS TO THE NATIONAL NEUROLOGICAL RESEARCH BANK-HUMAN NEUROLOGICAL SPECIMEN BANK

Q. When should arrangements be made for organ donation

A. Now. If you have been told by your doctor that you have a neurological illness such as multiple sclerosis, Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), epilepsy, or a psychiatric condition, we must still carefully document your medical history by consultation with your physician. If you do not have any neurologic or psychiatric illness but are still interested in the organ donation program, other aspects of your medical history, treatments, etc., must also be documented. Early arrangements simplify enormously the task of being sure of your medical history. In addition, an early donation makes it much more likely that your family will become aware of your desire to participate and be prepared to cooperate with your wishes at the necessary time.

Q. Can I donate brain and neurological tissue to the National Neurological Research Bank and the rest of my body to a major medical center as an anatomical gift?

A. In general, this cannot be done. The majority of anatomical gift programs are intended to provide general educational training for medical students and do not provide for partial donations. The National Neurological Research Bank serves as a repository for research into specific neurological and psychiatric disorders. The Gift of Hope Organ Donation Program provides the opportunity to contribute directly to research efforts aimed at the prevention, treatment, and cure of people with similar illnesses. If you are already a member of a general anatomical gift program but would like further information about changing to a donation to the National Neurological Research Bank Gift of Hope Program, we are available to answer your questions. You may, however, belong to other anatomical gift programs such as those involving donation of eyes and/or kidneys, as well as belonging to the National Neurological Research Bank donation program.

Q. Will the organ donation affect any family plans for funeral and burial procedures?

A. No. Performance of an autopsy will not interfere with the funeral plans. An autopsy is an operation which leaves no scars visible during a normal funeral procedure. In addition to providing donated tissue to the Research Bank, the autopsy may help the family by supplying a definitive cause of death, may define further insurance benefits due the family, and may show the presence of infections or inherited diseases, in which the case the family can be informed and the proper precautions taken.

NATIONAL NEUROLOGICAL RESEARCH BANK

Authorization for Autopsy and Retention of Tissue for Research

With the desire to contribute to furthering medical knowledge and progress

I myself

I/we being the next of kin or legally authorized representative(s)

Spouse Sister

Daughter Person entitled by law to control the remains of the prospective donor/patient

Son

Brother

Hereby authorize a post-mortem examination immediately following

my ___ his ___ her

death including the removal and retention or use of such organs, tissues and specimens as deemed necessary for scientific research purposes.

Special instructions (if any)

Note: All signatures must be original

Signature of Donor or authorized representive

Signature of Witness (required)

Date

Relationship of witness to Donor

Ratified following death of donor on ___, 19___ at

by ___ telephone ___ telegram ___ in person

by

NOTE: COPY OF AUTOPSY AUTHORIZATION MUST ACCOMPANY ANY SPECIMENS SENT TO BANK

NATIONAL NEUROLOGICAL RESEARCH BANK

ORGAN DONATION PROGRAM

KEEP ON FRONT OF PATIENT'S CHART

Patient Name ___ Date ___

Social Security Number ___

This patient may be participating in an organ donation program (O.P.D.) for research purposes. If this patient becomes critically ill or dies, the following persons should be notified immediately

1. Primary physician
See Neurology OD Schedule for doctor on call

AND

		Day	Nights/Weekends/Holidays
2	Robert Baumhefner, M.D. MS Fellow	Ext 2504 Pager 426	339-9770
	W.W. Tourtellotte, M.D. Chief, Neurology Service	Ext 5113, 4171	454-7763
	Malu Bird, R.N. Co-Director of O.D.P.	Ext 5113, 4171	208-7657
	Diane Guntrip Administrative Assistant Neurology	Ext 5113, 4171	390-5448
	Bank Technicians		
	Keith Berman	Ext 5113, 4171	552-3053*
	Larry Domoto	Ext 5113, 4171	826-5805
	Jim Kelly	Ext 5113, 4171	399-8239

3. Next-of-kin (to obtain autopsy permission)

Name

Phone

Address

*Weekends only

3/25/81

When a patient decides to donate their brain tissues to the National Institute Research Bank they are given information flyers explaining the program and a card to carry in their wallet. This card alerts emergency personnel to perform autopsies expeditiously in case of accidental death.

NIH Photo 1779

Feel free to write or call the NINCDS-supported brain banks for any information you may need, as well as for the donor forms and cards. The addresses and phone numbers are:

W.W. Tourtellotte, M.D., Director
National Neurological Research Bank (127)
VA Wadsworth Medical Center
Los Angeles, California 90073
(The following numbers may be called collect.
Ask for Dr. Tourtellotte, or leave a message and your call
will be returned.)
Brain Bank: (213) 824-4307 or FTS 794-4171
VA Hospital: (213) 478-3711, ext. 4171. If no answer at extension, ask operator to call Dr. Tourtellotte at home. (Phone answered 24 hours a day.)

Edward D. Bird, M.D., Director
The Brain Tissue Resource Center
McLean Hospital
Belmont, Massachusetts 02178
(617) 855-2400 (This number may be called 24 hours a day, collect.) ■

Interferon Produced in Patient with Rare Leukemia

Prepared by the National Institute of Dental Research Information Office

Scientists from the National Institutes of Health (NIH) and the Walter Reed Army Medical Center have discovered that white blood cells (lymphocytes) from a patient suffering from a rare leukemia can spontaneously produce interferon. The study of this case may provide clues into how interferon regulates some of the complex functions of the immune system.

Interferon was first recognized over 20 years ago as a protein that prevents viruses from growing. Recently, scientists have shown that there are three general classes of interferon called alpha, beta, and gamma. These interferons have other biological effects other than interfering with viral growth. They can inhibit cell multiplication, suppress tumor growth and affect cells of the immune system.

The immune system consists of a variety of cells that interact with each other in a complex way. These interactions are regulated by a variety of factors including interferon. Diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis are characterized by immunologic abnormalities.

Several years ago, Dr. John J. Hooks, at the National Institute of Dental Research (NIDR), found that interferon was present in the blood of patients with some of these immunologic diseases, while it is not present in normal individuals. In fact, in patients with SLE there is a correlation between disease activity and the presence of interferon in the blood. Thus, interferon is thought, somehow to be involved in this disease. Scientists do not know whether it is one of the causative factors or one of the host's defenses against SLE.

In the latest issue of *Blood*, the Journal of the American Society for Hematology, Dr. Hooks and coworkers describe a patient with a white cell proliferative d ease (chronic lymphocytic leukemia) whose lymphocytes spontaneously produce immune (gamma) interferon, a phenomenon that has not previously been recognized.

Gamma interferon is the most active of the interferons in regulating the immune system and can even potentiate the biological actions of the other interferons. Finding a cell programmed to produce gamma interferon may enable scientists to use cell cloning and recombinant DNA techniques to obtain sufficient quantities of this interferon to evaluate its role in clinical studies.

The studies by Drs. John J. Hooks, Barton F. Haynes, Barbara Detrick-Hooks, Louis F. Diehl, Theresa Gerrard and Anthony S. Fauci of the National Institute of Dental Research, National Institute of Allergy and Infectious Diseases and the Walter Reed Army Medical Center were reported in the January 1982 issue of the Journal *Blood*. ■

More Research Needed to Decide on Benefits of Restrictive Diets for Hyperactive Children

Prepared by the National Institute of Child Health and Human Development Information Office

Special restricted diets, which eliminate artificial food additives and some natural chemicals, should not be used universally to treat hyperactive children until more research is done. That was the basic conclusion of a 13-member expert panel assembled recently at the National Institutes of Health to assess the effectiveness of diet in treating hyperactivity.

Following a three-day consensus development conference, the 13-member panel released a statement January 15 saying that restricted diets should be considered, along with all other possible therapies, only after a thorough evaluation of the child and his family. The task force noted that such dietary restrictions appear to help a "small group" of hyperactive children but said that current scientific evidence is insufficient to identify which children may be in this group.

Citing a lack of information in many critical areas, the statement called for expanded and improved research into the causes of hyperactivity and the various approaches to treatment, including diet. The panel suggested establishing a set of standard diagnostic criteria, examining risk factors for the condition, and developing ways to predict which children might respond to a given therapy. Also needed, the panel added, are more concise ways to measure variables such as attention span, learning ability, and social adjustment.

In situations where a restricted diet is recommended by a physician, the panel said special attention should be given to individual cultural, ethnic, and socioeconomic factors to ensure proper implementation of the diet. The panel also stressed the importance to consumers of having all ingredients in food products properly labeled.

Hyperactivity is generally characterized by excessive motor activity, short attention span, and impulsive behavior for a child's age. The panel, made up of physicians, scientists, consumers, and an attorney, said the syndrome appears in more boys than girls, the actual number of school-age children affected is unknown: estimates range from 1 to 20 percent. Traditional therapy for the condition has included medication, behavioral modification, educational techniques, and psychotherapy.

A dietary approach to treatment began in 1973 with reports of clinical success by a San Francisco allergist, Dr. Ben F. Feingold. His regimen eliminated "processed" foods with artificial flavors, coloring, and most preservatives, as well as foods such as almonds, cucumbers, tomatoes, apples, and berrries which contain high levels of natural chemicals called sodium salicylates.

At the NIH meeting, Dr. Feingold said these dietary restrictions may help up to 50 percent of all hyperactive children. However, the panel chairman, Dr. Floyd Denny of the University of North Carolina, said the percentage of children responding is probably much lower, although there is no way to identify who would be likely to respond.

The consensus panel found that, wh dramatic improvements have sometime been observed during uncontrolled clinical trials, controlled scientific studies of the effectiveness of restricted diets have produced inconsistent results. In other biologic studies that have looked for possible adverse effects of food additives, only red dye No. 3 has been shown to sometimes cause increased motor activity in animals. The panel concluded that more research is needed on the potential positive and negative effects of the dietary approach.

Discrepancies between clinical observations and much of the available scientific evidence prompted the conference, sponsored by the National Institute of Allergy and Infectious Diseases, the National Institute of Child Health and Human Development, and NIH's Office of Medical Applications of Research.

Consensus development conferences are designed to bring together biomedical investigators, practicing physicians, consumers, and advocacy groups to discuss, assess, and agree on the safety and effectiveness of particular technologies, including drugs, devices, procedures, and treatments.

For the full consensus statement, contact the Office for Medical Application Research, Bldg. 1, Rm. 216, Bethesda, MD. 20205, or call (301) 496-1143. ■

Editors of publications may request a glossy print of the numbered photographs appearing in this issue.

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National Institutes of Health
Building 31, Rm. 2B-10
Bethesda, Maryland 20205

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News&Features from NIH

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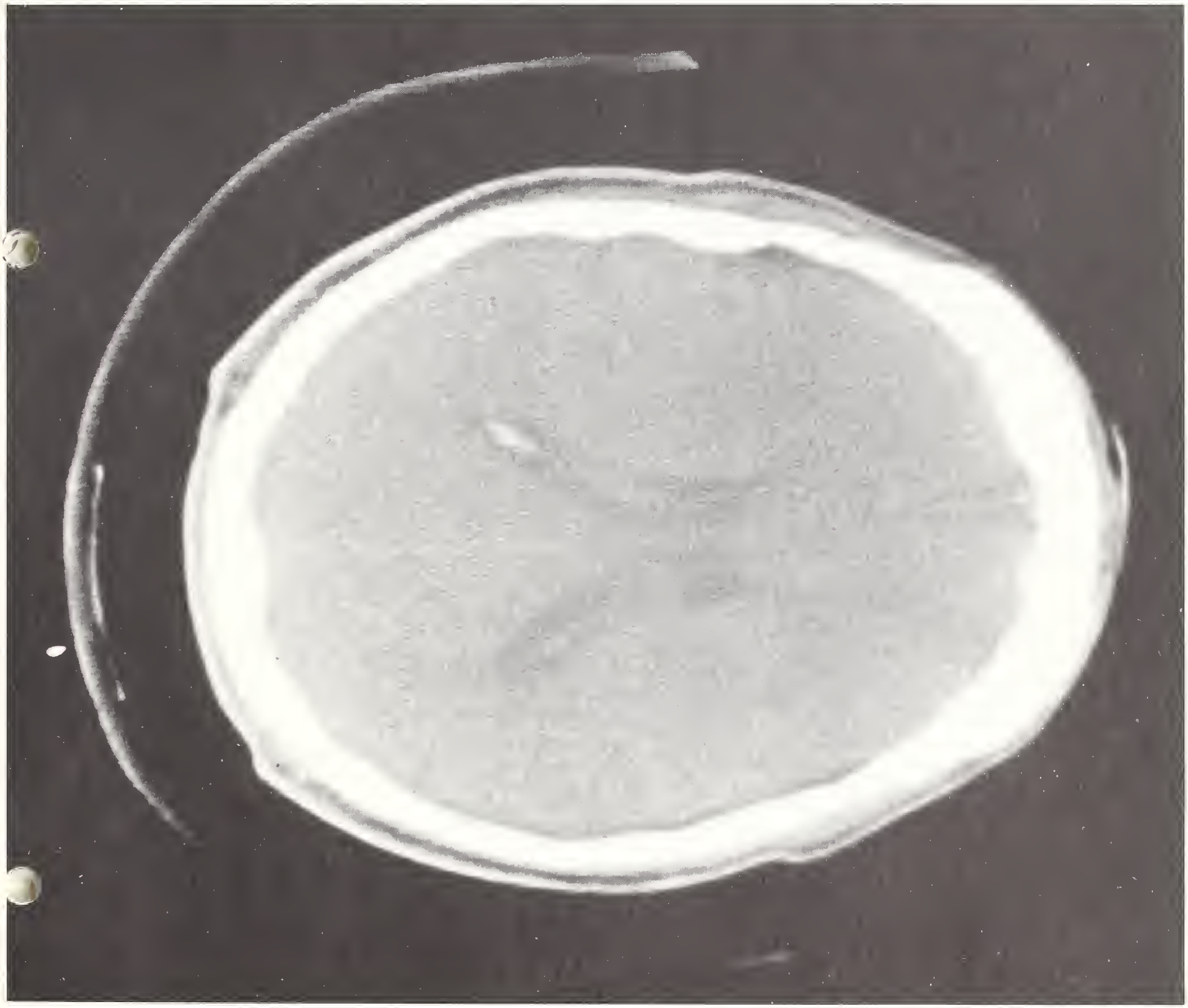

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NIH Photo 1780

News&Features
from NIH

April 1982

Measles, a childhood illness that can cause pneumonia, blindness, deafness, and sometimes encephalitis, was the topic at a recent international meeting held in Washington, D.C.

Scientists from all over the world discussed the impact of measles, present control programs . . . and nationwide vaccination programs.

At the conclusion, the world physicians agreed that total eradication could be achieved probably within the next two decades. See page 4.

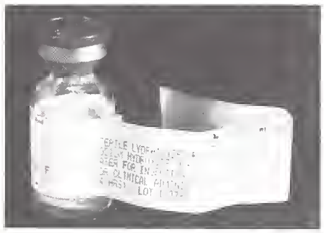
"JC" a name for a virus that causes a rare neurological disease, was recently discussed at an NIH-sponsored science writers seminar.

This virus causes progressive multifocal leukoencephalopathy (PML) in patients whose immune systems are weakened by cancer or autoimmune diseases. The patient, once stricken, appears to be suffering from a stroke, but as the virus progresses, the disease begins to affect the nervous system and eventually the patient becomes blind and demented. See page 9.

An NIADDK research chemist recently developed a synthesized derivative of opium, allowing the U.S. to become less dependent on foreign suppliers of opium for medicinal uses.

The process invented by Dr. Kenner C. Rice, provides the simple and practical total synthesis of three opioid substances. See page 11.

Researchers at NIAID have found that the drug, acyclovir, prevents the recurrence of herpes simplex infections. The drug inhibits viral shedding and causes few side effects. See page 3.



NIH researchers are testing synthetic growth hormone—made from recombinant DNA research—on patients with growth hormone deficiency. Two dozen children will receive the hormone and their growth rates compared to those of children who received the natural product. If all goes well, the hormone may be commercially available in a few years. See page 5.

Puberty—the physical and psychological development of boys and girls, sometimes affects children in infancy instead of at the normal age of 11 to 13. This accelerated growth was once treated with a drug that causes several side effects, but now scientists at NICHD and the Massachusetts General Hospital have devised a new treatment to slow down sexual development. See page 5.

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Editorial Staff

Mary Donovan
Editor

Susan Gerhold
Associate Editor

Helen Ou
Special Assistant

Maureen Napper
Editorial Assistant

NIH News Branch
Building 31, Room 2B-10
Bethesda, Maryland 20205
Phone:
(301) 496-2535

Nuclear Magnetic Resonance: The Chemist's Tool Enters the Brain Age

Ray Fleming

The sign on the door reads simply "NMR Room," and any industrial research chemist could tell you about the machinery inside that analyzed complicated molecular structures. After all, NMR, or nuclear magnetic resonance, has been a standard technique used for decades in chemistry to identify and characterize different liquids and solids.

Today this same NMR technique, combined with technology derived from computed tomography (CT) scanners, is beginning to produce remarkable anatomical and metabolic pictures of the human brain and spinal cord. And the continuing development of this chemist's tool as an emerging brain imaging technique is "capturing the excitement of many people in neurobiology," according to Dr. Murray Goldstein, acting director of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS).

At the heart of the NMR technique are the characteristic energy signals released by individual atomic nuclei when these particles are made to vibrate or resonate in a magnetic field disturbed by radio waves. If placed in the tunnel-like core of an NMR machine's large electromagnet, the human body will release these same energy signals from the nuclei of its tissues when bursts of radio waves are sent through the magnetic field. Each signal varies according to the particular nucleus that generated it (hydrogen, phosphorus, or sodium, for example), and the characteristics of the tissue surrounding that nucleus. In the NMR imaging process, each signal is measured and assembled with other signals by computer to produce an image resembling a CT scan. From this image, scientists can determine tissue density and the distribution of different elements within the body.

Images of the body (particularly of the brain and spinal cord) emerging from the handful of NMR scanners now being developed herald the technique's potential for studying the nervous system. Signals from the individual nuclei yield images that are especially clear and detailed and by manipulation of the NMR system, cross-sectional pictures in almost any plane and two- and three-dimensional images are possible. "I am astonished," says Dr. Juan Taveras, professor of radiology at Harvard Medical School and an NINCDS grantee, "at the degree of resolution already obtained. Anatomy can be shown beautifully by NMR.

Because white matter, for example, produces a range of energy signals different from those in the cerebral cortex, these two types of tissue can be distinguished in an NMR image. More importantly, however, especially for studying neurological and communicative disorders, an abnormal cerebral cortex produces signals different from one which is normal. Comparison of normal and diseased tissue images will be an important factor in future confirmation of nervous system disorders.

Certain biochemical differences become well defined when imaged on the NMR screen. The technique distinguishes well between gray and white brain matter, an ability that scientists hope will aid in the study of demyelinating and sclerosing disorders. Tumorous and dead tissue can be identified, and constricted blood vessels that may lead to stroke are apparent in some scans. Excellent images of bony structures, such as the spinal column, can be readily produced by NMR.

The technique's inherent ability to assimilate information from individual atomic particles may also make it useful for studying basic biochemical processes. Measurement of blood flow in the brain and other areas of the body is a possibility, and some investigators have demonstrated that NMR analysis of phosphorus in the body may allow them to better understand metabolic processes. Phosphorus, although present in only limited amounts in the body, is an important component in the ATP (adenosine triphosphate) cycle, the biochemical pathway from which the body draws its energy.

Although nuclear magnetic resonance is still a developing technology, the procedure is considered safe; no invasive methods or ionizing radiation are involved. NMR, however, does have drawbacks. The magnetism generated during scanning can disturb the operation of certain medical equipment in the immediate area, notably instruments employing cathode ray tubes. Small metallic objects such as scissors and stethoscopes can actually be drawn toward the magnetized area. Dr. Taveras explains that patients with metallic implants may be unsuitable for NMR scanning, and points out that "some people even develop claustrophobia" when placed in the magnet's tunnel-like central core.

Inevitably, the advantages and disadvantages of any new technology invite comparison with other existing systems. With the development of such successful brain imaging systems as computed tomography and positron emission tomography (PET), why the need for NMR? The answer is that although there is some overlap in the imaging abilities of these systems, each measures a different component of the livng brain: CT shows tissue density, PET measures biochemical activity over time, and NMR gives information about the molecular makeup and concentration of atoms in the tissue. When all three technologies are readily available (CT scanning is currently the only system in widespread use), scientists expect each to contribute in its own way to improvements in the diagnosis, treatment, and prevention of neurological and communicative disorders.

Ultimately, NMR may have more far-reaching effects on medical research than either CT or PET. Because NMR is capable of imaging any area of the body (depending on the size of its magnetic core), its potential goes well beyond the brain and spinal cord. "In the chest [illegible] heart," says Dr. Taveras, "the potential is tremendous. I have a feeling that NMR may prove even better from the head down than from the neck up."

Further research in the coming decade by physicists, biologists, and engineers will undoubtedly define the limits of this technique. For now however, NMR—the chemist's tool— is adjusting comfortably to its new role as a window for the human body, particularly the brain. ■

Acyclovir: Effective in Preventing Recurrent Herpes Infections

Joan B. Hartman

A research group at the National Institute of Allergy and Infectious Diseases (NIAID) has shown that the antiviral drug, acyclovir (ACV) prevents recurrence of herpes virus infection.

Using intravenous and oral acyclovir (ACV) supplied by the Burroughs-Wellcome Corporation, Dr. Stephen Straus and his colleagues in the Medical Virology section, NIAID, were able to treat successfully over 25 episodes of recurrent herpes infection in five patients. The ACV rapidly inhibited viral shedding caused few side effects, and permitted complete healing of lesions. Four patients had genital herpes and one had facial herpes.

As expected, the ACV did not prevent later recurrences once treatment was discontinued. Also, once discontinued, it did not postpone the onset of recurrent symptoms past the interval seen between naturally-occurring episodes. However, using long courses of oral ACV at lower than treatment level doses, Dr. Straus was able to suppress symptomatic recurrences of infection for as long as administration of the drug continued (up to 65 days).

Side effects were limited to redness and irritation at the intravenous puncture site in two patients (due to improperly situated intravenous catheters); several bouts of diarrhea and nausea in one patient; a mild tremor in one patient; a twofold rise in one patient's liver enzyme levels, which returned to normal within one week after completion of treatment; and, a temporary drop in one patient's white blood count, which also returned to normal once ACV was discontinued.

The research group monitored the patients frequently for the possible development of drug-resistant virus strains and found none. However, Dr. Straus emphasizes that, "repeated ACV treatments favor selection of drug-resistant virus strains." He suggested that chronic therapy might be preferable to intermittent therapy for individuals who suffer frequent, severe recurrences. Virus then would not have the opportunity to repeatedly reactivate and be exposed to suboptimal drug concentrations.

Conversely, Dr. Straus has not endorsed long-term therapy for most individuals: "It is our feeling that the development and understanding of antiviral drugs and their potential toxicities has not yet grown sufficiently to permit us to recommend very long term suppressive regimens."

Acyclovir, one of the new drugs of the 80s, seems to show promising results in preventing recurrent herpes infections.
NIH Photo 1781

To learn more about the drug and its potential for long term effects, Dr. Straus recently has undertaken a limited, double-blind placebo study with 32 "normal" (immune competent) patients who have severe recurrent genital herpes. Each of the 16 men and women selected have experienced at least 12 episodes annually for a duration of one year or more. The study will last for four months.

Dr. Straus underscores that the treatment is still experimental and cannot be recommended for most normal persons with mild or intermittent episodes of disease.

The report of this research was the lead article in the March issue of the *Annals of Internal Medicine*.

Coauthors of the paper are Holly A. Smith and Chaim Brickman, M.D., both of Medical Virology, NIAID, and Paulo DeMiranda, Ph.D., Colin McLaren, Ph.D., and Ronald A. Keeney, M.D., all of the Burroughs-Wellcome Corporation, Research Triangle Park, N.C. ■

Eradication of Measles Planned at International Meeting

Mary Donovan

Panelists represented countries from all over the world, at the recent International Symposium on Measles Immunization.

NIH Photo 1782

Worldwide eradication of measles can be done through local control programs and international cooperation was the general agreement reached by scientists from 21 countries, including the United States, at a 4-day meeting held last month and planned by the Fogarty International Center. The session took place at the headquarters of the Pan American Health Organization in Washington, D.C.

The International Symposium on Measles Immunization was convened to assess the impact of measles as a cause of childhood illness and death around the world. Contrary to popular opinion, measles is not an innocuous disease, but can cause pneumonia, blindness, deafness and, in some cases, encephalitis. Worldwide it kills 1.5 million children annually.

Dr. Saul Krugman from the New York University Medical Center and the symposium chairman, opened the meeting by stating, "We have made great strides in controlling measles since the first international symposium on measles in 1961. Vaccines—available since 1963—have sharply reduced the number of measles cases and death."

"Measles eradication is unlikely unless world leaders are committed to primary health care for all their citizens, especially for poor women and children," said Dr. Ralph Henderson of the World Health Organization to symposium participants. He underscored the need for support for the WHO's campaign for Health for All by the Year 2000. Its goal is to have all people at a level of health where they can lead socially and economically productive lives.

He further stressed, "Immunizations are the most inexpensive and most effective interventions we have. Measles immunization can be provided for as little as 12 cents per child."

Although the participants felt that the developed countries must assist the less wealthy nations, the essential burden rests with individual countries to provide local control before global eradication can be achieved.

In the U.S., vaccine use has reduced the incidence from 500,000 to 3,000 cases annually. According to Dr. Alan Hinman of the Centers for Disease Control, "this is because more than 90 percent of U.S. children are immunized for measles as part of the mandatory school entry requirements in each state."

"Except for import cases, added Dr. Hinman, it is very likely that measles will be eliminated by October 1982 in this country."

Currently, Czechoslovakia, Albania, and Costa Rica also have successful measles vaccination programs, but in many other nations the extent and success of their programs have been, at best, variable. Poor motivation and a lack of awareness on the part of public health officials have been the major barriers in industrialized countries, especially in Western Europe. In developing nations, limited resources and manpower have severely restricted the effectiveness of their eradication efforts.

Symposium participants agreed on the safety and effectiveness of the currently available vaccines, which are made from live, attenuated viruses. One dose, usually adminstered near the child's first birthday, is generally sufficient for protection. In tropical countries, delivering a potent vaccine is complicated by the warm climate which makes refrigeration difficult.

Since measles is so contagious, the strategy for eradication is to immunize as many infants and young children as possible. Several speakers emphasized t although the goal is similar, the appro is different from the smallpox eradication program which aimed at immunizing specific populations.

As summarized by Dr. Samuel Katz from Duke University, "There is no reason why successful measles immunization can't be extended to all the world's children. The challenge before us is to exploit available vaccines and to overcome the remaining economic, logistical and attitudinal barriers."

No exact time was set for eventual eradication, but some participants asserted that it is achievable within the next decade or two.

In addition to the Fogarty International Center, the meeting was cosponsored by the National Institute of Allergy and Infectious Diseases, the National Institute of Child Health and Human Development, the Centers for Disease Control, the Bureau of Biologics, the U.S. Agency for International Development, the World Health Organization, the Pan American Health Organization, the Institute of Medicine of the U.S. National Academy of Sciences, the American Public Health Organization, and Merck and Company. ■

Recombinant DNA Product Tested at NICHD

Susan Johnson

In one of the first medical applications of recombinant DNA research, scientists at the National Institute of Child Heath and Human Development (NICHD) and nine other medical centers are testing synthetic human growth hormone in patients with growth hormone deficiency.

About 20,000 children in the United States fail to grow at a normal rate because they do not produce enough growth hormone. This hormone, made by the pituitary gland, is the body's main growth-promoting agent.

In most cases, growth hormone deficiency occurs for no obvious reason. Among the known causes are tumors, infection, radiation to the head, and head injury. The disorder can also be inherited.

The patient being treated at NICHD apparently lost the ability to make sufficient growth hormone as a result of an accident 4 years ago in which she fell and hit her head. Until that time, her father recalls, she and her identical twin sister had always been the same height.

After the accident, the patient's growth slowed, while her sister continued to grow at a normal rate. Today, at age 12, she is 4 feet 3 inches tall, four inches shorter than her sister and below normal for her age.

Treatment for growth hormone deficiency consists of replacing the hormone. Until now, the only source of human growth hormone has been the pituitary glands of cadavers. Supplies have therefore been scarce.

The synthetic growth hormone now being tested is a product of genetic engineering, a technology developed only 9 years ago. Scientists at Genentech Inc., a South San Francisco research firm, prepare the hormone by inserting the human gene for growth hormone into the DNA, or genetic material, of bacteria. As the bacteria multiply, they produce large amounts of human growth hormone.

Preliminary tests in animals showed synthetic growth hormone to be nontoxic and as effective as natural hormone in stimulating growth. The safety of the synthetic hormone in humans was demonstrated this past fall in a test involving a group of normal, healthy adults.

The effectiveness and safety of synthetic growth hormone in children with growth hormone deficiency is now being tested in patients at NICHD and nine medical schools. A total of two dozen children throughout the country will receive the hormone during these trials.

Researchers will compare the growth rates of the children to those achieved in the past with natural growth hormone. If all goes well, synthetic growth hormone may be available commercially within 2 to 3 years.

The biggest impact of synthetic growth hormone will be in overcoming the shortage of the hormone for research and medical treatment, says Dr. Barry Bercu, a pediatric endocrinologist at NICHD.

With unlimited supplies of growth hormone now becoming available, researchers can try the hormone in children who are short for reasons other than growth hormone deficiency—a population estimated at 100,000 in the United States. NICHD is planning a study this spring, says Dr. Bercu, in which synthetic growth hormone will be tested in children who are short for various reasons, including delayed puberty and an inherited tendency towards shortness. ■

Brain Hormone Halts Premature Sexual Development

Susan Johnson

A potential new treatment for premature puberty has been discovered by a team of scientists from the National Institute of Child Health and Human Development (NICHD), the Salk Institute, and Massachusetts General Hospital. They found that large doses of a brain hormone that normally triggers puberty can halt, and even reverse, sexual development.

The new treatment promises to be safer and more effective than the currently available therapy for premature puberty, says Dr. Florence Comite, an NICHD member of the research team.

Puberty normally begins around age 11 in girls and 12 or 13 in boys. In rare instances it starts much sooner, even in infancy. This can result from recognizable abnormalities in the central nervous system, but often there is no identifiable cause.

Premature puberty disturbs both the physical and psychological development of its victims. Children with the disorder develop sexually mature bodies and often undergo the emotional upheaval and sexual awakening of adolescence before they are ready for these changes.

Patients who enter puberty prematurely are tall for their age as children, because of accelerated growth associated with sexual maturation. They are frequently short as adults, however, because they complete their growth at an earlier than normal age.

Previously, the only treatment for premature puberty was a synthetic progestin. This drug stops menstruation, but usually fails to prevent the rapid growth that results in short adult stature. Treatment with synthetic progestin can produce several serious side effects.

Physicians at NICHD and Massachusetts General Hospital have devised an alternative treatment for premature puberty that interferes with the chain of hormonal events necessary for sexual maturation.

Puberty begins when the brain starts secreting a hormone called luteinizing hormone-releasing hormone (LHRH) in periodic bursts. LHRH stimulates the pituitary gland to release hormones called gonadotropins. These hormones, in turn, stimulate the gonads (ovaries and testes) to make sex hormones, which cause sexual maturation.

The rate at which the brain releases LHRH is crucial to sexual development. Unless LHRH is secreted intermittently, the pituitary gland will not release enough gonadotropin to stimulate production of sex hormones. Research has shown that continuous administration of LHRH desensitizes the pituitary gland to the hormone, causing the pituitary to stop releasing gonadotropins.

The physicians took advantage of this phenomenon to inhibit gonadotropin secretion in five girls aged 2 to 8 who had started puberty. They gave the girls a modified form of LHRH, supplied by the Salk Institute, that is more potent and longer-acting than the natural hormone. Daily injections of the LHRH analog have the same effect as continuous administration of LHRH.

After 2 months of therapy, gonadotropin and sex hormone levels had dropped to prepubertal levels in all five patients. Treatment reversed breast and pubic hair development in one of the girls. None of the patients menstruated during therapy.

Treatment with the LHRH analog did not produce any serious side effects, and its therapeutic effects were reversible. Two months after treatment was stopped, the patients' gonadotropin and sex hormone levels had returned to pretreatment levels.

Although this study included only girls, more recent work at NICHD has shown that LHRH analog therapy for premature puberty is also effective in boys, says Dr. Comite. She and her coworkers are currently studying the effects of long-term treatment with the LHRH analog on growth and eventual sexual development. Preliminary results show that therapy slows growth to normal prepubertal rates.

The use of an LHRH analog to treat premature puberty was reported in a recent issue of the *New England Journal of Medicine* by Drs. Comite, Gordon B. Cutler, Jr., and D. Lynn Loriaux of NICHD; Drs. Jean Rivier and Wylie W. Vale of the Salk Institute; and Dr. William F. Crowley, Jr., of Massachusetts General Hospital. ■

Image Processing Aids Researchers in Quest for Information

Dale Blumenthal

National Institutes of Health researchers are collaborating with computer specialists, using methods called "image processing" to extract and analyze information in photographs, chromatograms, and images recorded by scientific instruments.

The computer acts as an extension of the eye and brain. It picks out information the scientists cannot see.

Jim DeLeo is one of several people in NIH's Division of Computer Research and Technology (DCRT) who have been working with researchers from several NIH Institutes, assisting them in analyzing their images with the computer.

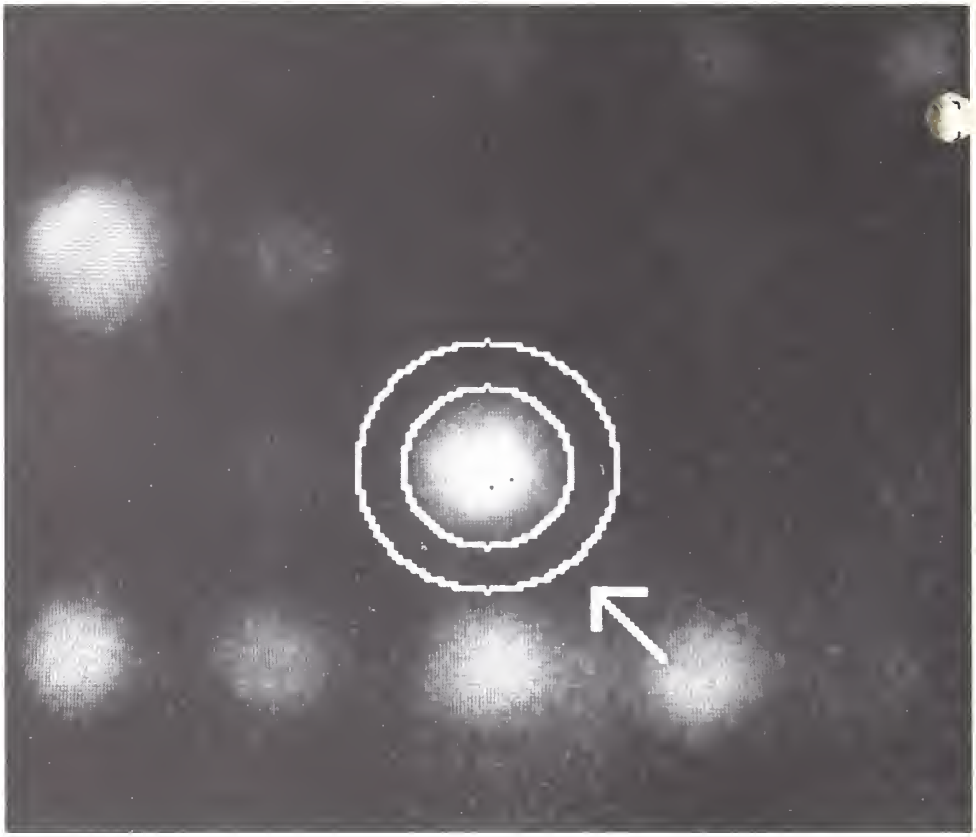
For instance, DeLeo is working with Dr. Floyd Taub, an NCI Research Associate. Dr. Taub is studying genetic changes in liver cells that occur in response to the steriod drug, dexamethasone. He uses the computer to determine how much the hormone affects specific DNA sequences.

Taub locates reactions with hybridization techniques and then records the results with a process called autoradiography. Autoradiography produces 96 spots on film, representing reaction sites for 96 DNA sequences. Then, Taub uses a microdensitiometer to measure the varying densities of the spots and translate them into numbers for the computer. The computer displays the image on a video screen, showing 96 circular spots at once. The color intensities are added to highlight the different amounts of reaction,.

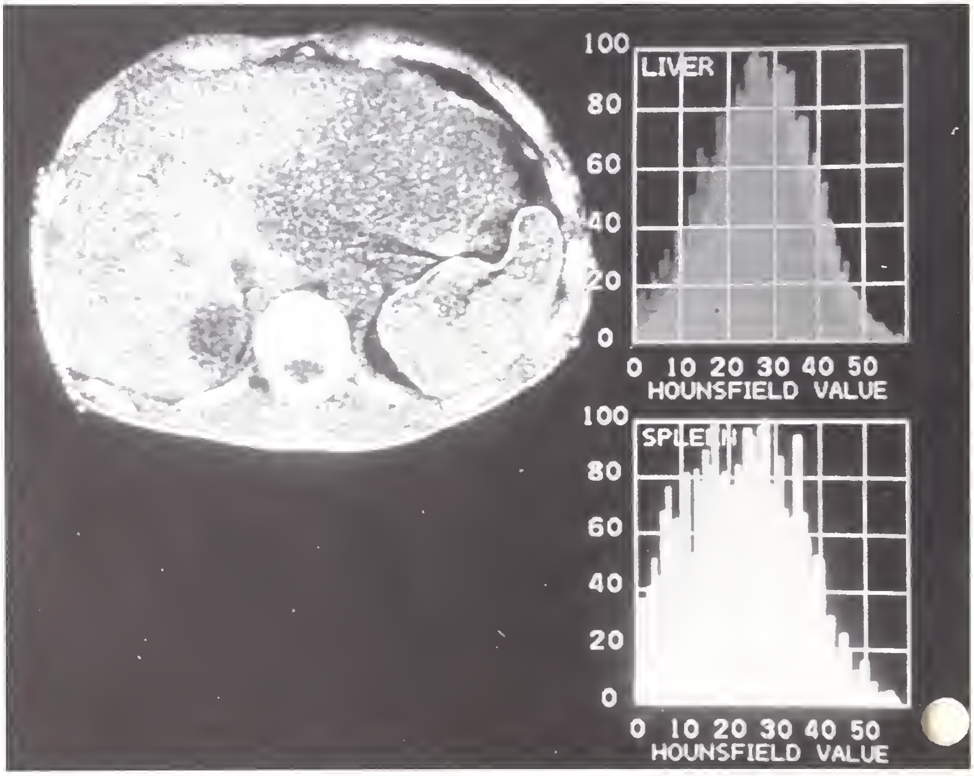
Taub points to a spot with a graph pen, and the computer calculates the amount of reaction. "Think of each spot as a mountain," he says. "There are three concentric circles within each mountain. The inner ring is the peak, and the largest circle is the background." He moves to a spot, and the computer calculates the size of the whole mountain above the background.

It takes only a few minutes to measure each set of 96 autoradiographic spots. "It would take a week without image processing," Taub says as he surveys the entire picture. Now he can study DNA sequences en masse.

In a project quite different from Taub's, researchers are using image processing to study the relationship between images from Positron Emission Tomography (PETs) and Computer Assisted Tomography (CT).



The inner circle points out the "peak" amount of hormonal reaction in this autoradiographic spot, and the outer circle outlines the "outside" amount of reaction. **NIH Photo 1783**

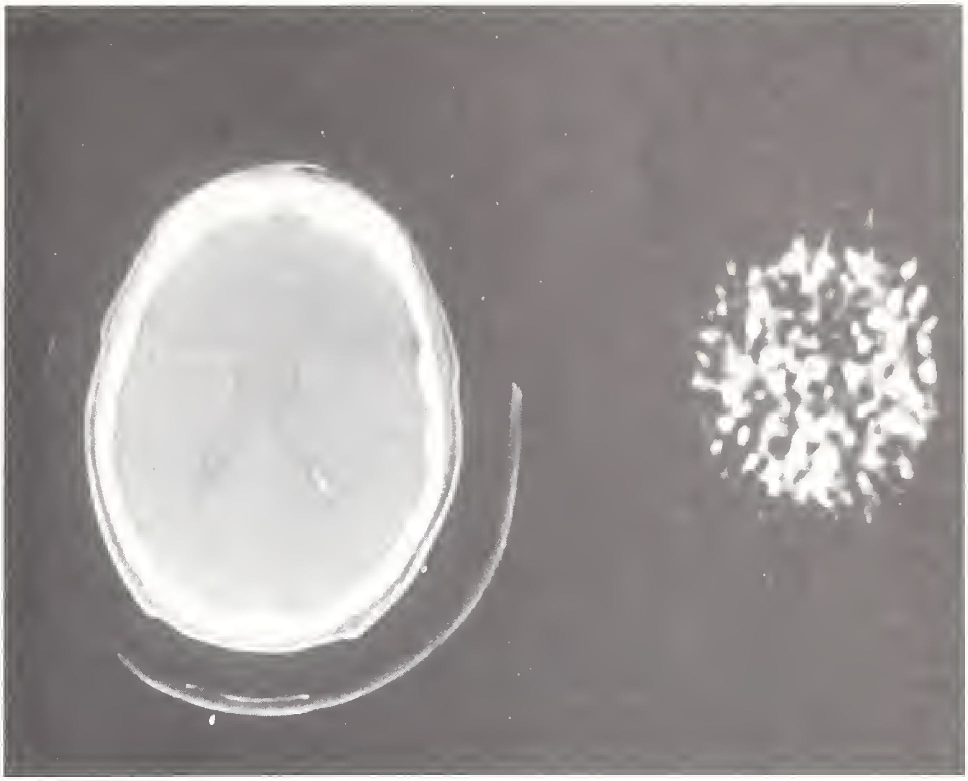


These histograms are actually multicolored images on DeLeo's computer screen. They compare densities in the scan (left) for before and after injection of contrast material.
NIH Photo 1784

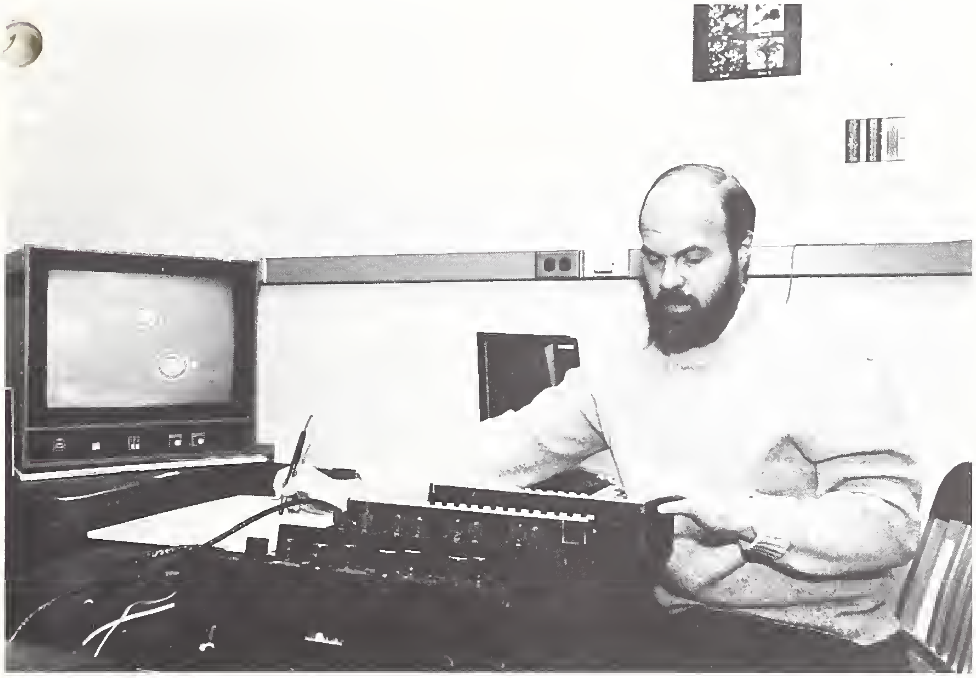
A CT scanner is a sophisticated machine which uses x-rays to show internal structures. A PET scanner records the emission of short-lived radioactive isotopes injected into the patient. While the CT scanner shows anatomy, the PET scanner can be used to reflect metabolic activity of the brain.

At the National Institute on Aging (NIA), scientists are looking at biochemical events in specific regions of the PET scanner records, by specifying regions in CT scans superimposed over corresponding PET scans. DeLeo is working with NIA scientists to measure and analyze the computerized images, in order to understand better the structure and function of changes in the brain associated with the aging process.

DeLeo is also working with ophthalmologists from the National Eye Institute (NEI) and at Harvard to use the computer to make measurements from photographs of slit-lamp images of the eye lens. Slit-lamp photography is a procedure ophthalmologists rely on to characterize the extent and location of cataracts. Although imprecise, clinicians analyze the results subjectively, recording notes on what they see. "Development of an objective contract grading scheme is a high priority of cataract researchers," states DeLeo.



Scientists would like to use computers to correlate CT scan images (left) with PET scan images (right). **NIH Photo 1785**



Jim DeLeo uses a graph pen to isolate DNA sequences in Dr. Floyd Taub's hybridization study, so that he can measure the amount of hormonal reaction. **NIH Photo 1786**

With a microdensitometer, or video techniques, they produce a digitized version of the photograph and enter it into the image processing computer system. The computer produces a histogram of density values with computed statistical measurements. This enables scientists to quantitatively assess cataract distribution. They anticipate that this method will be useful in timetracking the progress or recession of cataract disease and in evaluating therapies.

DeLeo describes other image processing projects on which some of his DCRT colleagues have worked. Each study is an example of a new purpose for image processing, and together they show the scope of this technique's usefulness. A project recently completed is the computer analysis of plaque formation in arteriosclerosis.

Scientists at the National Heart Lung, and Blood Institute (NHLBI) photographed sections of the arterial trees of experimental animals fed different diets. The animals were of different sizes and species. Computer specialists helped them devise a way to produce a standardized image for each particular grouping of animals. Together the team of physicians and computer specialists could measure disease incidence at a given location in the standardized image, using colors of various intensities to represent levels of plaque deposits. They drew topographic maps, correlating data on anatomy, diet, and vessel wall permeability. DeLeo describes this project as significant for characterizing pattern and distribution of plaque formation in the arteries.

Both researchers and computer specialists agree that image processing can augment existing technologies with speed and precision. Scientists can then analyze their images, using the computer to bring out even more information.

New possibilities depend on insight and imagination and on new uses scientists see for these tools. ■

Mass Spectrometry: Weighing One Molecule at a Time

Hugh James Lee

The laboratory looks something like the inside of a submarine, packed with high technology instrumentation and a number of busy people, recognizable computer terminals and less familiar clusters of electronic gear. The scene is in the Analytical Chemistry Group laboratory at the National Institute of Environmental Health Sciences (NIEHS) located in the pine forests of Research Triangle Park in North Carolina.

The "stars of the technological show" in the lab are four mass spectrometers, instruments that can identify and determine levels of chemicals in samples down to incredibly small concentrations of parts per trillion in some cases. A fifth mass spectrometer is located at nearby University of North Carolina at Chapel Hill where NIEHS shares its use with university researchers. Although each instrument has its own personality and appearance, they all share the features of polished stainless steel, a multitude of control knobs and switches, and a complicated collection of connecting wires. The ticking of vacuum pumps and the gentle whine of cooling fans combine as background sounds in the laboratory.

The intensity of effort in the lab is immediately obvious to a visitor. As staff members set up and adjust the spectrometers there seems to be a rapport between the scientists and the electronic devices similar to that between musicians and their musical instruments—part harmony and part negotiation—as problems are traced and results pursued. Chemicals are introduced after evaporation into the high vacuum system into the ion source region of the instrument. Energy is added, converting sample molecules into changed ions. These are then subjected to various electric and magnetic fields which separate the ions on the basis of their mass per unit charge.

The group has adapted each of the four spectrometer systems in the lab to perform different specialized applications, and each system has what for a layman is a somewhat awe-inspiring name: ultra high resolution mass spectrometer; high resolution mass spectrometer; tandem quadropole mass spectrometer system; and the combined high pressure liquid chromotography system. The capabilities of these instruments stagger the imagination. An example of their highest performance can distinguish molecules which differ by one part in a million (i.e., mass 100.0000 from mass 100.0001). By appropriate sample manipulations, analysis of impurities at the part-per-trillion level can be performed.

To visualize this, it is like finding one faulty grain in 1,000 tons of wheat. When asked what he does, J. Ronald Hass, Phd., Head of the Analytial Chemistry Group, Hass simplifies his answer by saying that he weighs things, for it is by weight that all of the elements that make up more complex chemical molecules can be identified. If weights of molecules can be ascertained and the weights of fragments formed as the molecules come apart during analysis can be determined, the chemical composition and in some cases the molecular structure of a sample can be identified.

Though most work can be described as research, the Group has performed routine analysis in response to a number of environmental catastrophes. For example, when a chemical warehouse exploded in New Jersey, Dr. Hass and his staff determined the amount of the potentially poisonous dioxins that were captured in air monitoring equipment of a federal regulatory agency. In another instance they analyzed samples of orthochlorophenol for dioxins after a railroad accident caused a chemical spill in Missouri.

"These situations demonstrate the critical importance of accuracy," Dr. Hass says. "A mistake in the identification of a contaminant may lead to inappropriate action in response to a problem." In many cases mass spectrometry provides the only technology currently available with the capability to identify environmental chemical residues retained in the body from either one-time exposures or long-term, low-dose exposures that may cause disease. The goal of the group, as with the Institute, is to provide and support the basic research to identify agents of concern, how they are handled, stored and eliminated by the body, the specific organ or system they affect, and the diseas they cause. Learning this then facilita planning for proper handling and control and this assists in the prevention of environmentally related diseases.

James D. McKinney, Ph.D., Chief of the Laboratory of Environmental Chemistry comments: "I think we could say that we have one of the most diverse combinations of mass spectrometry equipment and capabilities under one roof in the United States. There is no question that this is a real national resource." Dr. Hass hastens to point out that the development of the Group to its present level has not been accomplished by free spending. "We have often had to barter additional floor space for instrumentation, and we have usually opted for instruments." When funds were not available for the store-bought version of the tandem quadropole system, the Group purchased key parts and, working closely with the Institute machinist Bob Hall, built the system themselves for about one-fifth the ready-made cost (excluding labor).

As the Group's reputation has spread at the Institute, it has been involved in three major activities: Support to biomedical research in areas as diverse as pharmacology and reproductive and developmental toxicology; development of analytical techniques; and basic resea in spectrometry itself. With these mult assignments, the Group currently has to limit its efforts to analysis in support of research conducted within the Institutes' own laboratories; they can therefore

Dr. Carol Parker (left) and Dr. Ron Hass conduct tests while developing new instrumentation.

NIH Photo 1787

accept no samples for analysis from outside sources that fall outside the boundaries of their existing research involvements.

During a typical year, the NIEHS spectrometers have been used for as many as 2.500 sample runs each. These runs conducted in addition to the research work of the staff include samples of human tissue, organ tissues from laboratory animals that have been exposed to environmental agents of concern, or chemicals analyzed to determine whether they are pure or contain any contaminants.

The particular spectrometry system selected to analyze a sample depends on the chemical's composition as well as the information that the researchers are trying to learn about the chemical. The Group is now modifying one component of its spectrometers, the ion source, to be able to use a method called Fast Atom Bombardment, or FAB for short. "With FAB we hope to be able to ionize larger molecules to enable us to look at higher molecular weights," Dr. Hass explains. "Large molecules are important in cancer research where it is critical to observe biopolymer chemical reactions."

The volume and sophistication of the group's work now depends on the efforts of nine full or part-time staff members. Besides Dr. Hass, permanent staff members are Carol E. Parker, Ph.D., a recognized authority on high pressure liquid chromatographic spectrometry who joined the lab in 1977; Donald J. Harvan, educated as an organic chemist and with the lab since 1976; and Michael Moorman, who joined the lab last year from another NIEHS laboratory where he played a key role in development of inhalation toxicology instrumentation. Former students working for the Analytical Chemistry Group currently hold positions such as Director of the Mass Spectrometry Group for the World Health Organization in Lyon, France; Research Assistant Professor, Department of Chemistry, Purdue University in Indiana; and Group Leader for Mass Spectrometry at the Frederick Cancer Research Center in Frederick, Maryland. "Training has been valuable to us, bringing in new ideas with fresh blood as well as giving us the opportunity to provide highly trained professionals for the biomedical research community," Dr. Hass says. He also notes that the lab has been able to attract distinguished Visiting Scientists who have made substantial contributions.

With the completion of a new NIEHS building in Research Triangle Park, the Analytical Chemistry group will remain in its old space but will inherit enough additional space from relocated labs to have a laboratory devoted entirely to spectrometry instrumentation. It is often true in the biological sciences that advances hinge on advances in technology and instrumentation. So it may be true that the chemists in the Analytical Chemistry Group will help in providing the key to unlock the future in biological advances.

"We want to improve the tools available to biomedical researchers, so new questions can be asked and the answers we can provide will be more precise and timely," Dr. Hass says. "We'd like to be able to work with smaller pieces of tissue samples and detect and identify chemicals at even lower concentrations." In the Analytical Chemistry Group at NIEHS, it appears the success story has just begun.

JC Virus: A Brain Tumor Model Linked to Rare Disease

Diane Striar

Chances are that lurking somewhere in your blood are antibodies for a virus called "JC." Scientists have detected these antibodies—the only evidence of an unrecognized, prior short-term infection by JC—in 84 percent of the U.S. population.

The National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) is studying JC because—for a small, unlucky group—the virus persists in the body, eventually causing a rare neurological disease. JC's other ability to cause brain tumors in animals provides one of the few model systems for the study of brain tumors in primates.

At a recent NIH science writers' seminar, NINCDS Infectious Diseases Branch chief, Dr. John L. Sever, and six scientists discussed current research on the biology of the JC-induced primate tumor, ways to diagnose the tumors, and progressive multifocal leukoencephalopathy (PML)—the rare disease caused by JC.

The virus was first isolated in tissue culture in 1971 when it was named for a PML patient whose initials were "JC."

"Primary infections with JC virus occur early in life in the majority of the population," said Dr. Sidney A. Houff, clinical neurologist with the NINCDS Infectious Diseases Branch. The symptoms of this primary infection and when it occurs are still unknown.

Years later, JC can strike again—this time in the form of PML. People whose immune systems are weakened by cancer, autoimmune diseases, or the use of immunosuppressive drugs after organ transplants are the most common targets of PML.

It is uncertain whether PML patients are experiencing a reactivation of the latent JC infection or are having an unrecognized primary infection, according to Dr. Houff. The role of the patients' deficient immune system in the development of PML is unclear too, he said.

Clinical symptoms of PML result from the JC virus' infection of the oligodendroglia cells. These cells make myelin, a fatty covering that insulates the axon or trunk of the nerve cell, and assists in the flow of messages sent along nerve fibers. When the oligodendroglia are infected, the myelin is destroyed in a process called demyelination that leads to the onset of symptoms.

Quite often the PML patient first appears to be a classic stroke case; but as the disease progresses, many areas of the nervous system are involved. Eventually the patient becomes blind and demented. Death usually results from infection.

Many PML patients have giant multinucleated astrocytes (cells that make up the supporting structure of nervous tissue) in their brains. These astrocytes have some malignant characteristics. Although several PML patients have also developed a type of cancerous brain tumor called an astrocytoma (grades 3 and 4 which are the most deadly kind); it is still not known whether the JC virus can cause malignant tumors in man. Research is underway to evaluate this possibility.

The JC virus has produced grade 3 and 4 astrocytomas in some nonhuman primates. Primates inoculated with JC virus who do not develop tumors may be protected from disease by their immune systems, said Dr. William C. Wallen, chief of the Viral Immunology Unit of NINCDS' Infectious Diseases Branch.

Dr. Wallen said he hopes that research on the role of immunity in monkey tumor growth will lead to the discovery of viral vaccines and tumor-related antigens that would prevent tumors.

Another possible benefit of the JC tumor research, according to Dr. William T. London, chief of the Experimental Pathology Section, is the development and evaluation of diagnostic tests for human use. Dr. London, who is conducting research on owl monkeys inoculated with the JC virus, noted that contrast-enhanced computed tomography (CT) allows for the detection of tumors while the animals are still clinically normal.

Like time-lapse photography, CT scans of monkey brains over several days show the rapidly developing tumors and such characteristics as tissue death and hemorrhage—even though the monkeys appear to be healthy. Similarly, CT scans of human brains are being used to predict pathological changes in a tumor.

Other aspects of JC viral research were presented at the science writers' seminar by Dr. Eugene Major, a visiting scientist with the NINCDS Viral Immunology Unit and Dr. Nancy Miller, an expert consultant to the unit. In addition, Dr. Clarence J. Gibbs, Jr., deputy chief of the NINCDS Laboratory of Central Nervous System Studies, discussed transmissible dementias and hemorrhagic fevers with renal syndrome. ■

Researchers Discover Bombesin, a Polypeptide, to Diagnose Tumors

Aubin Tyler and Hugh James Lee

A polypeptide, associated with an invasive human lung tumor, that might serve as a basis for early detection and treatment has been independently discovered by a team of investigators at the National Institutes of Health and at the Hammersmith Hospital in London, England.

At the National Institute of Environmental Health Sciences (NIEHS) scientists found bombesin, as the chemical is called, in small cell cancer experimentally propagated under the skin of nude mice.

They were using radioimmunoassay techniques to analyze and identify various peptide hormones in human small cancer and in carcinogen induced squamous cell cancer from mice and rats. According to Dr. Lawrence H. Lazarus, a research chemist at the NIEHS, "Bombesin immunoreactivity was initially found in 1978 in human fetal and neonatal lung tissue."

At the National Cancer Institute (NCI) and the National Naval Medical Center in collaboration with Drs. Terry W. Moody of George Washington University and Dr. Candace B. Pert of the National Institute of Mental Health, investigators report bombesin in cultures from 19 patients with small cell cancer of the lung (SCCL). Bombesin was not present in patients with other forms of lung cancer. In a few cases, high levels of bombesin were also found in the blood of patients with SCCL.

These data were further corroborated by Dr. Stephen R. Bloom in London who found substantial amounts of bombesin-like reactivity in small cell cancer resected patients and also reported low levels in normal lung tissue.

Although bombesin normally is plentiful in the brain, gut and fetal lungs, it is generally not present in the lungs of healthy adults. The scientists speculate that bombesin may sustain and promote tumor growth.

Small cell cancer of the lung accounts for one quarter of the 100,000 cases of lung cancer that are diagnosed annually. It is the most rapidly spreading of the four major types of lung cancer—which include epidermoid, adenocarcinoma, and large cell lung cancer—but it is also the most responsive to radiation and chemotherapy. Some 70 to 90 percent of patients with SCCL respond to therapy, but the majority of cases are far advanced at the time of diagnosis, with spread of the cancer to the brain, liver or bone. Relapse is commonplace. If SCCL is not treated, the median survival time is 56 days. Those patients who respond to treatment have a medium survival of 10 months; only about five percent of patients with the disease live beyond the two-year mark.

The outlook for patients with non-SCCL is somewhat better, because their cancers tend to be confined to the chest and to grow more slowly. Patients with non-SCCL are not treatable with radiation or anticancer drugs, but about 25 percent of them can be treated with surgery. Those patients who are successfully treated surgically have an 80 percent five-year survival rate. Untreated patients with non-SCCL have an average survival rate of two to three years.

The unique appearance of bombesin in SCCL tumors but not other lung cancers holds promise of a new diagnostic tool. If the peptide could be readily detected in the blood or urine of persons at high risk for lung cancer, such as smokers, earlier diagnosis and higher survival rates might become possible. Such a marker might also be used to monitor patient response to treatment.

Although a few patients with extensive SCCL had elevated blood levels of bombesin in the NCI study, the peptide is difficult to detect in blood because it is quickly destroyed by enzymes in the blood. However, careful collection and storage of blood samples and the use of enzyme inhibitors may improve the technique to measure bombesin levels in blood. The scientists are currently studying the role of bombesin blood levels in the staging of SCCL patients and in evaluating their response to therapy.

In laboratory cell culture, the NCI researchers found that SCCL cells actively released bombesin which, in turn, encouraged the growth of those cells. If this is the case, an agent that could block the activity of bombesin might represent a mechanism to control tumor proliferation in SCCL patients.

Bombesin levels may be associated with an array of physiological effects, such as loss of appetite and subsequent weight loss, altered sugar metaboli and lowered temperature in SC patients. If bombesin levels could be controlled, related symptoms in those patients might be held in check.

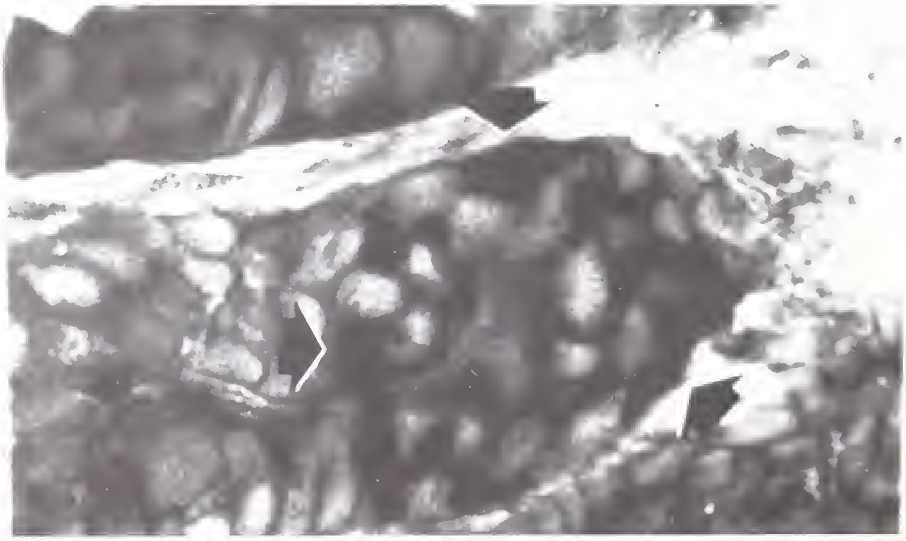
Another potential application of bombesin specifically may be in the typing and classification of lung tumors. Microscopic examination of tissue is often uncertain in the case of lung cancers and may delay appropriate treatment. Prompt identification of SCCL, based on the presence of bombesin in tissue specimens, could make pathology studies more precise and serve as a guide to selection of therapy. A study is under way to review lung tissue specimens of 100 patients to test this hypothesis.

The scientists believe that SCCL may arise from a specific subset of lung endocrine (hormone-producing) cells which proliferate after exposure to carcinogens. Studies of rodents exposed to carcinogens (asbestos, diethylnitrosamine) showed these cancer-promoting substances to induce the growth of endocrine cells, including bombesin-producing cells, in the lungs.

Additional studies underway are attempting to find the exact location and chemical identification of bombesin tumor cells.

The research at NCI and the National Naval Medical Center was reported in the December 11, 1981 issue of *Science* by Drs. Adi F. Gazdak, John D. Minna and Desmond N. Carney.

The work at NIEHS will be published in the April 1982 issue of the *Proceedings of the National Academy of Science* by M.D. Erisman, R.I. Linnoila, O. Hernandez, R.P. Di Augustine and L.H. Lazarus. ■



Immunoreactive bombesin (see arrows) are seen in cells of human lung small cell carcinoma.

NIH Photo 1788

Synthesis of Medical Opiates Developed by NIADDK

Linda Cross

A research chemist at the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases has developed a process for the total synthesis of medically important opium derivatives.

A significant advance toward the large-scale synthetic manufacture of these drugs, the new method may provide future independence from foreign suppliers of opium, currently the sole U.S. source of medical opiates. In addition, this process will ensure the availability of quantities of unnatural opioids needed as research tools.

The process, invented by Dr. Kenner C. Rice, provides the first relatively simple and practical total synthesis of three important opioid substances, dihydrothebainone, dihydrocodeinone, and nordihydrocodeinone, both in their natural and unnatural forms.

These intermediates can be transformed into natural morphine, codeine, thebaine, and all other medically valuable opiates and their unnatural isomers. Using existing practical methods.

Valuable contributions in this area were made by Drs. Henry Rapoport and D.D. Weller of the University of California at Berkely, who described highly efficient conversion of dihydrothebainone and dihydrocodeinone to codeine and thebaine several years ago.

About the same time, Dr. Rice discovered a rapid, high yielding method for synthesis of morphine from codeine, which until then, had been a longstanding problem in the chemistry of the opium alkaloids.

The medical importance of codeine alone, one of the most commonly prescribed analgesic and antitussives in the world, is reflected by its rising annual U.S. production and use, in excess of 55,000 kilograms, and worldwide production, 190,000 kilograms.

In developing an efficient process to synthesize unnatural opiates, Dr. Rice simultaneously obtained a method that could be adapted for the total synthesis of all naturally derived opiates as well.

Synthesis of a number of unnatural opiates, which is being funded by the National Institute on Drug Abuse, is now in progress at Research Triangle Institute under direction of Drs. F.I. Carroll, G.A. Brine and A.H. Lewin. Patent applications protect the United States' interests in the new process, and all rights have been transferred from the Department of Health and Human Services to the Department of Commerce.

Dr. Rice was recently recognized for this invention at the 9th Annual World Fair for Technology Exchange in Atlanta, Georgia, where he was awarded second prize in the Government Division.

The new process is another milestone in a research effort that has spanned centuries, originating with discovery of raw opium as a folk remedy and leading toward the practical total synthesis of its important alkaloids, morphine, codeine, and thebaine, and their derivatives.

Opium, which is the dried sap of the unripe seed capsules of the opium poppy, has been cited since Homeric times as a painkiller. It is believed that its remarkable pharmacological effects were recognized as early as 3500 B.C.

With the evolution of alchemy into chemistry and medicine in the 18th and 19th centuries, scientists began examining folk remedies to determine their active chemical components. Opium was the first material to be analyzed successfully when it yielded crystalline morphine in 1805.

After the isolation of this alkaloid by a German pharmacist, F.W.A. Serturner, scientists were encouraged to isolate and describe other pure components in opium as well as in other plant-based remedies. This effort ultimately led to the chemical characterization and synthesis of other active agents in many naturally-occurring materials.

By 1925 the gross structure of morphine had been elucidated by Sir Robert Robinson, but it was not until 1952 that Dr. Marshall Gates of the University of Rochester, New York, announced the first total synthesis of this major opium alkaloid. During the same period, the last details of its chemical structure were finally settled by classical chemical degradation.

Subsequently, seven other groups of scientists synthesized morphine or intermediates that had been converted to morphine by following several conceptually different pathways. While each was of considerable scientific significance, none of these expensive and time-consuming processes was of practical importance.

Consequently, morphine and codeine are still manufactured from opium or poppy straw concentrate imported from abroad, a situation that could change as a result of the work of Dr. Rice. Production of opioids in the U.S. by cultivation of the opium poppy is not permitted by law. ■

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News&Features from NIH

May 1982

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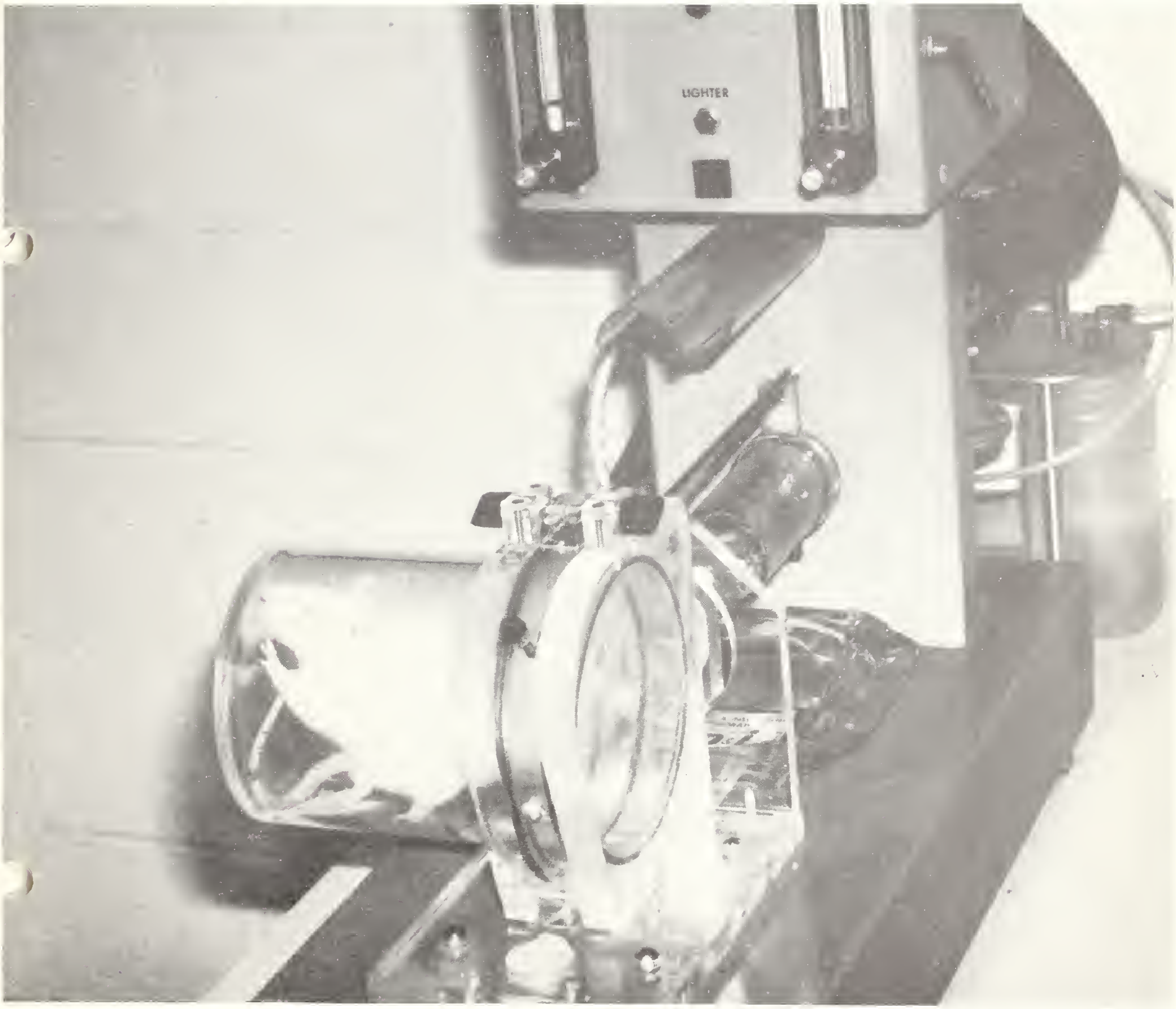


U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

NIH PHOTO 1789

News&Features from NIH

May 1982

NCI researchers are currently studying two families, one living in Virginia, and one in North Carolina, both with an abnormally high occurrence of cancer. The two families are not related, but do share common bonds—smoking and occupation. The NCI investigating team hopes that focusing their research on these families, and their common bonds, will help to identify markers of risk for lung cancer and other types of cancer found in families throughout the U.S.

See page 7.

NIGMS grantees are currently studying a protein, that produces a fever during infection, and may also be responsible for elevating body temperature during exercise. If true, this theory may explain the claim that regular exercise wards off infection.

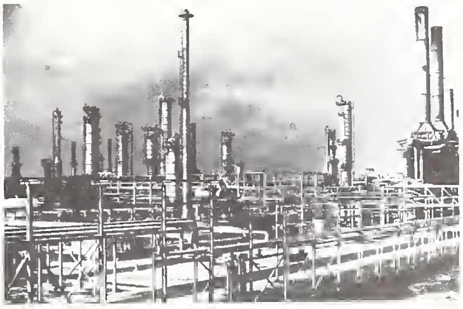
Some investigators suggest that a rise in temperature during exercise represents a resetting of the body's thermostat. When this happens, the body undergoes physiological changes and builds up a resistance to bacterial growth, which may help prevent infection.

See page 11.

Petroleum workers were recently studied by NCI and NIOSH investigators to find out why employees in oil refinery industries seem to develop more cases of brain and stomach cancers, leukemia, multiple myeloma, pancreatic, prostate, and skin cancers.

Future studies are planned to try to identify work environments that place employees at a higher risk for developing certain types of cancer.

See page 6.



NIH PHOTO 1790

Propranolol, a beta-blocking heart drug, does lower the mortality rate of heart attack victims, says the NHLBI in their final report of the Beta-Blocker Heart Attack Trial (BHAT).

Over 30 clinical centers throughout the U.S. and Canada participated in the trials. Patients were selected by age and had suffered at least one myocardial infarction (MI).

The latest data reveals that those patients treated with Propranolol had less chance of suffering from arteriosclerosis or sudden death from cardiovascular disease.

See page 4.

Rats were used as an animal model to identify whether smoking causes fetuses to be smaller and underweight at birth and whether organs other than the lungs are affected by maternal smoking.

Researchers noted that not all parts of the fetus'body are affected equally. The lungs seem to be more affected than other organs.

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Editorial Staff

Mary Donovan
Editor

Susan Gerhold
Associate Editor

Helen Ou
Special Assistant

Maureen Napper
Editorial Assistant

NIH News Branch
Building 31, Room 2B-10
Bethesda, Maryland 20205

Phone:
(301) 496-2535

Researchers Identify First Biochemical Cause of Male Infertility

Susan Johnson

The first known biochemical cause of male infertility has been identified by a team of scientists from the National Institute of Child Health and Human Development (NICHD), Laval University, and the Population Council. They found that an enzyme deficiency causes some men to produce nonmotile sperm.

The underlying cause of most male infertility is poorly understood. A man's ability to become a father depends largely on whether or not his sperm are functioning properly. Sperm function, in turn, depends on biochemical processes about which little is known. The new finding will aid our understanding of the biochemical basis of male infertility, says Dr. Richard Sherins, the NICHD member of the research team.

A major part of sperm function is motility. Sperm must swim to reach and fertilize an egg. Scientists know little about the mechanisms that control this purposeful movement. Some men who are infertile because of low or absent sperm movement have sperm with structural defects. The sperm of most of these men, however, appear normal structurally.

Recent studies have shown that an enzyme called protein-carboxyl methylase (PCM) is involved in controlling the movement of bacteria and certain white blood cells. The testes, which produce sperm, have a high concentration of this enzyme, and all the components for PCM activity are present in the tail of mature sperm. These considerations suggest that PCM is involved in regulating sperm motility.

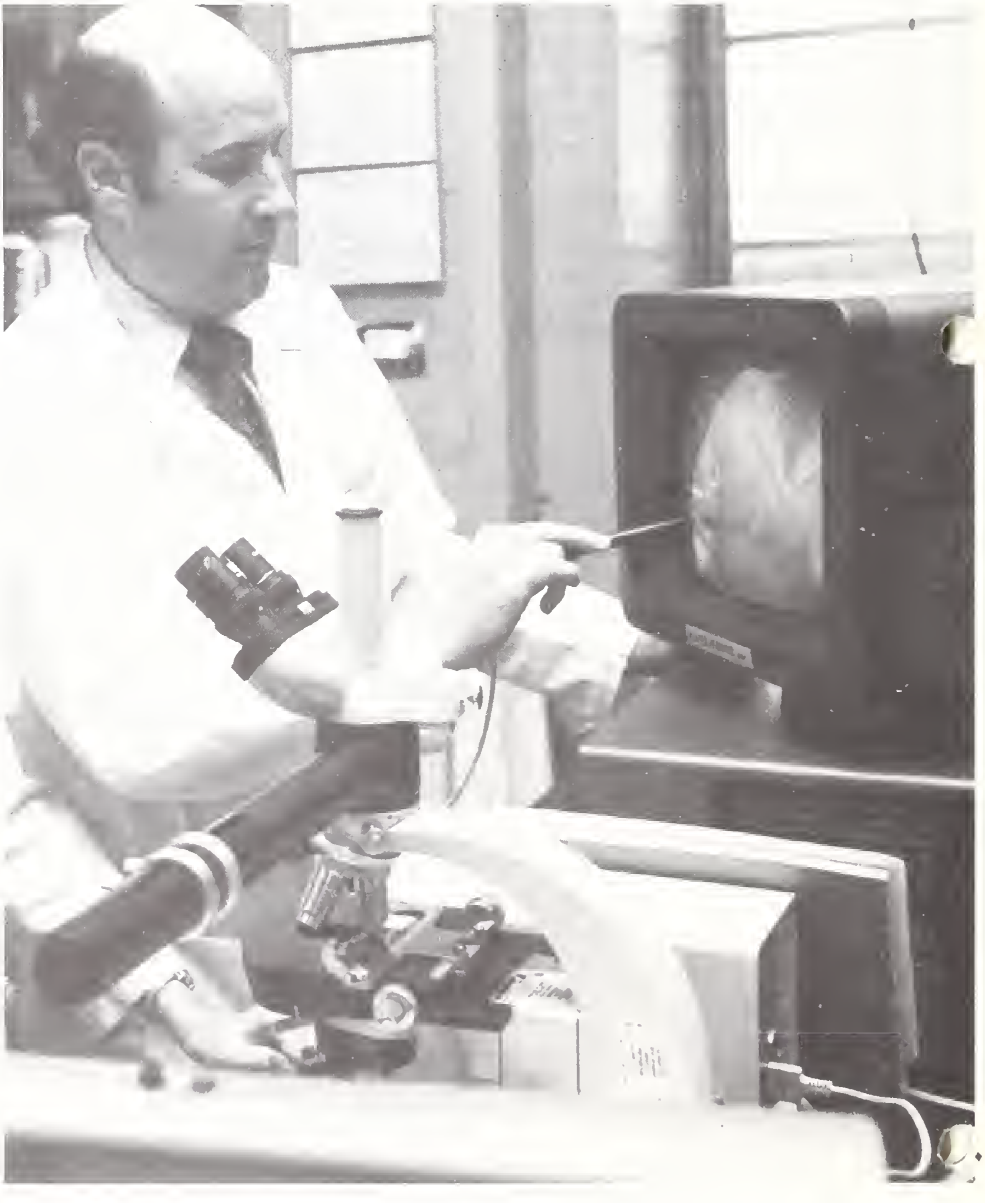
To see if this is the case, the scientists looked at the levels of PCM activity in the semen of three groups of men: 22 normally fertile men, ten vasectomized men, and nine patients with infertility caused by sperm nonmotility. They found that PCM activity in the semen of the infertile patients was only one-fourth that of the fertile men, and was similar to PCM activity in the semen of the vasectomized men, which contained no sperm.

The researchers concluded from these findings that PCM is involved in controlling sperm movement, and that PCM activity in semen is concentrated mainly in the sperm. Their conclusions are supported by the recent finding that substances which inhibit PCM activity also hinder sperm motility.

The cause of the PCM deficiency in the infertile patients is unknown. Through biochemical tests, the researchers ruled out the possibility that low PCM activity was the result of a high number of dead sperm in the semen or of leaky sperm membranes, through which PCM could escape. Using an electron microscope, they also showed that low PCM activity in semen was not associated with structural abnormalities in sperm.

PCM is not the only mechanism controlling sperm motility, the researchers noted. Most likely, sperm movement is regulated by a series of biochemical reactions. Elimination of any one of these reactions might result in infertility.

This study was reported in the April 8, 1982 issue of the *New England Journal of Medicine* by Dr. Claude Gagnon of Laval University. Dr. Sherins, and Drs. David Phillips and C. Wayne Bardin of the Population Council at Rockefeller University. ■



Dr. Richard Sherins of NICHD and a scientific team from the Population Council and Laval University, have recently found that an enzyme deficiency causes some men to produce nonmotile sperm. This information will help the investigators to better understand the biochemical basis of male infertility.

NIH PHOTO 1791

NCI Investigators Find Cell Receptors Involved in Cancer Process

Lorraine M. Kershner

Scientists at the National Cancer Institute (NCI) have identified receptors on cell surfaces that occur naturally but also may play a role in transformation of cells to cancer. Dr. Peter M. Blumberg reported at a recent meeting of the American Association for Cancer Research in St. Louis, that some tumor promoters act by binding directly to specific receptors on the surfaces of cells.

Tumor promoters are substances that do not cause cancer by themselves but complete the process of carcinogenesis in cells already exposed to low doses of other cancer-causing agents. Because the effects of a promoting substance on the cell are reversible, finding ways to remove or inhibit such a substance might be important for cancer prevention.

Using an assay developed in his laboratory, Dr. Blumberg and colleagues measured the binding of phorbol esters—a common class of tumor promoters that occur naturally in some plants—to cells from different tissues of mice and other animals. They found that these chemicals bind to the cell membrane at specific sites or receptors. This finding is important because it suggests that this binding triggers these receptors which in turn mediate key biochemical processes in the cell.

Other evidence supporting a key role for the receptors is their wide occurrence throughout the animal kingdom. Not only are they present in similar levels in all vertebrate species examined—including man—but they occur as well in invertebrates, such as worms, sea urchins and fruit flies. This suggests that their function is important to the functioning of the cell, Dr. Blumberg said.

Further study suggested to the NCI scientist that these receptors are probably a cellular protein that functions as an enzyme, as part of the cell's structure, or as a component of the systems that transport other biochemicals within the cell. The NCI researchers are currently working to identify this protein. Learning how promoters influence the normal protein to subvert a basic cellular process might provide scientists with valuable information on the basic mechanism of promotion.

The process Dr. Blumberg discussed is part of the now classic two-stage mechanism of carcinogenesis first described by Dr. Isaac Berenblum in the 1940's. Dr. Berenblum found that certain chemicals—which he called tumor promoters—could not themselves cause cancer but could greatly enhance the tumor producing effects of carcinogens that he called initiators. When he applied a single low dose of a carcinogen to a mouse's skin, the mouse developed few tumors. He then applied a variety of irritants to the same area of the skin. Many carcinogens irritate tissue, and Berenblum wanted to see whether irritation per se had anything to do with carcinogenesis. Although most of the irritants had no effect, one—a phorbol ester called croton oil—greatly increased the number and size of tumors that formed. Alone, though, croton oil caused no tumors.

Since Berenblum first described the phenomena, much has been learned about tumor promotion. Scientists now know that the process is not limited to skin. A number of experiments indicate that promotion is important in the development of cancers in many organs, including lung, colon, bladder and liver in a number of animal species. Also the list of substances suspected of being promoters has expanded to include the drug phenobarbital, the artificial sweeteners saccharin and sodium cyclamate, the hormone estrogen, and a component of agent orange, among others. ■

Activating Normal Genes may Explain Transformation of Normal Cells to Cancer

Lorraine M. Kershner

One of the fundamental question in cancer research—what causes a normal cell to lose control and turn into a cancerous one—is closer to being resolved. The answer may lie in the activation of a few genes present in normal cells. These findings were discussed by National Cancer Institute scientist George F. Vande Woude at the 73rd annual meeting of the American Association for Cancer Research in St. Louis, Missouri.

Most of the present understanding of the role of these normal cellular genes comes from the study of a group of tumor viruses (retroviruses) that possess cancer genes, also called *onc* genes. These *onc* genes are hardly unique to retroviruses. Within the last few years, scientists have found that closely related copies of many of these genes are present in normal cells of all vertebrates studied, from fish to humans.

This discovery suggests that over the course of time, viruses became cancer-causing by capturing cell genes and incorporating them into viral hereditary information. In normal cells, these genes probably govern some important functions in the cell's life, perhaps related to the control of growth and differentiation. They also are likely to be closely regulated by the cell, expressed some of the time, but repressed at other times.

A virus carrying stolen genes, however, can subvert this tight control when it invades a host cell by inserting the genes into places where they are no longer properly controlled. This may trigger biochemical changes within the cell that lead to uncontrolled growth.

Prior to studies by Dr. Vande Woude and his colleagues, it was uncertain whether *onc* genes occurring in normal cells were structurally similar to the *onc* genes carried by viruses. More important, it was not known whether the cellular *onc* genes could cause normal cells to transform into cancer cells.

The NCI scientists began their studies by first isolating a mouse retrovirus from its host cell chromosome. They showed that the isolated virus could transform mouse cells efficiently. They then dissected the virus with recombinant DNA techniques to determine which of its region or regions was responsible for the transforming activity. These studies revealed that only two regions of the virus were necessary: the viral *onc* gene and the viral transcription control element (called the long terminal repeat (LTR)).

Dr. Vande Woude and his colleagues then isolated the *onc* gene from normal mouse cells. Alone, this gene could not transform cells, but, if they added a portion of the viral LTR to the normal cellular gene, it activated its ability to transform cells. Again, only two elements were necessary for transformation: the normal *onc* gene and the LTR transcription control element carried by the virus.

At least three other laboratories have been able to activate a normal cell *onc* gene by inserting or attaching a viral transcription control element. Chromosomal translocations, rearrangements, or mutations resulting from a variety of insults to a cell's DNA could potentially cause transformation by juxtaposing a transcription control element adjacent to a normal cell gene with transforming potential.

Scientists now are searching for the expression of known retroviral *onc* genes in human cancers. In other animals, they have identified at least 15 of these genes. Many of these genes are similar.

If some of the viral *onc* genes are responsible for human cancers, it would be possible to detect their protein product and we would immediately have a diagnostic tool, Dr. Vande Woude said. In the long run, scientists hope that by identifying tumor-specific *onc* genes and their protein products, we will ultimately learn how to cure and prevent cancer. ■

Final Results In—Propranolol Reduces Deaths from Second Heart Attacks

Prepared by the National Heart, Lung, and Blood Institute

Propranolol, known to many as Inderal, is a beta-blocking drug used for patients suffering from heart attacks.

After careful study, the National Heart, Lung, and Blood Institute announced that this drug can reduce mortality from recurrent heart attacks within the first 12 to 18 months following a heart attack. Other results show that Propranolol also protects heart attack patients up to a full 39 months following an attack. NIH PHOTO 1792

Photo compliments of Ayerst Laboratories.

Final results of the National Heart, Lung, and Blood Institute's (NHLBI) Beta-Blocker Heart Attack Trial (BHAT) indicate that treatment of heart attack patients with propranolol can reduce mortality from recurrent heart attacks.

These results verify the preliminary findings (published in November 1981) that heart attack patients given propranolol had a 26 percent lower mortality rate than did similar patients who were given placebo during the average follow-up period of 25 months. The benefits of propranolol were not affected by the patient's age or site of infarction.

The trial involved 32 clinical centers and one coordinating center in the U.S. and Canada. Patients were selected between the ages of 30 and 60 years, and had experienced a documented myocardial infarction (MI). More than 16,000 patients in the clinical centers met the criteria, but many were excluded because of a history of asthma or low blood pressure that could cause potential contraindication to the drug. In all, 1,916 patients were enrolled into the propranolol group and 1,921 patients were randomized into the placebo group.

Treatment of the patients began five to 21 days after they experienced a heart attack. Propranolol was administered orally three times a day in doses of 180 mg or 240 mg. The placebo medication also was divided into three daily doses.

After leaving the hospital following the heart attack, patients were asked to report to the clinical center one month later, followed by a second visit six weeks later and then at three-month intervals thereafter.

Data generated by the study were reviewed by members of the Policy and Data Monitoring Board, not BHAT investigators. The board met seven times during the study to review the mortality data and consider the probability of a statistically significant treatment being considered at the conclusion of the study. The board concluded that propranolol therapy was effective and recommended that BHAT be terminated nine months earlier than originally planned. (June 1982) Based on this recommendation, patient follow-up ended October 2, 1981, and preliminary results of the trial were announced at a press conference at NIH on October 29. The final data offers information on disease—specific causes of death, which the initial announcement was able to offer.

The data show that, after an average follow-up of 25.1 months, 138 (7.2 percent) of the propanolol-treated group and 188 (9.8 percent) of the placebo-treated group had died. Most of the deaths were related to cardiovascular disease; only 11 of the propanolol group and 17 of the placebo group died of causes not related to cardiovascular problems.

Propanolol was found to have reduced mortality from arteriosclerotic heart disease (6.2 percent among the propanolol group va. 8.5 percent among the placebo group) as well as sudden death from cardiovascular disease (3.3 percent of the propanolol patients vs. 4.6 percent of the placebo patients). Differences in mortality rates for all cardiovascular causes proved statistically significant between the two group of patients.

Whether the patients had had a single uncomplicated myocardial infarction, a myocardial infarction with complications or had had more than one myocardial infarction, propranolol proved to be effective. Side effects from the drug were infrequent and usual—encountered with beta-blocking drugs. Some complications (depression, nightmares) usually attributed to the use of this class of drug were reported as frequently among the placebo group as among the propranolol group.

The results of the BHAT study reflected those from other studies using beta-blockers in patients who had had a heart attack. The Norwegian Multicentre Study Group trial of Timolol (which is presently the only beta-blocker that the FDA has approved for use in the U.S. to prevent recurrent heart attacks) and a more recent study using metoprolol also yielded positive results.

None of these studies was designed to answer the question of how long after a myocardial infarction the treatment with the beta-blocking agent should continue. Nor is it known whether initiating treatment a substantially longer time after the heart attack will provide equal benefit. The efficacy of propranolol was most pronounced in the first 12 to 18 months following the heart attack, although the benefits could be seen for the full 39 months of maximum patient followup.

In conclusion the Research Group stated that, "based on the BHAT result, and in conjunction with those of studies reported previously, the investigators recommend the use of propranolol for at least three years in patients with no contraindications to beta-blockade who have had a recent MI."

For more information contact National Heart, Lung, and Blood Institute, Public Inquires and Reports Branch, Building 32, Room 4A21, NIH, 9000 Rockville Pike, Bethesda, Maryland 20205. ■

Changes in Cell Surface Components may Explain Why Some Microbes Cause Disease

Linda Stalvey

NIH investigators have discovered a mechanism that may help explain the virulence of certain bacteria and viruses. Dr. Loretta Leive and coworkers at the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases have shown for the first time that bacteria, whose cell surface component activates complement poorly, escape being eaten by macrophages and therefore cause disease.

Salmonella typhimurium is the organism that was used in these experiments. Like other bacterial strains, the degree of its pathogenicity is determined by cell surface components. One of the most important determinants of virulence is lipopolysaccharide.

Previous investigators had shown that by changing genetically that polysaccharide portion of the LPS, bacteria can be made less virulent. Depending on which polysaccharide replaces the original one, the bacteria can be rendered 10 or even 100 times less pathogenic.

While the effect of this change was clear, the mechanism of action remained obscure.

Dr. Leive, in conjunction with Mrs. Y. Takasaki of Japan and in collaboration with Dr. P.H. Makela of Finland, has now shown that the change in polysaccharide changes the rate of phagocytes (or ingestion) of the bacteria by white blood cells called macrophages. Phagocytosis changes because each polysaccharide activates complement proteins differently. Complement is the name given to a group of blood proteins that act together as part of the body's immune system. This group of proteins participates in the phagocytosis process, and in other biologic processes that help defend the body from infection.

One of these proteins, called C3, "coats" the activating particle or microbe with a protein fragment that is recognized by macrophages as a signal for ingestion. Therefore, macrophages will ingest the bacteria whose polysaccharide activates complement poorly.

To show that phagocytosis was indeed involved in the difference in pathogenicity, macrophages were allowed to ingest three different strains of *Salmonella*, each differing only slight in the polysaccharide of the LPS. The rate of ingestion was inversely proporational to the known virulence of the bacterial strains: the most virulent was taken up the slowest, the intermediate strain was taken up at an intermediate rate, and the least virulent was taken up the fastest. The differences in ingestion rates reflect the affinity of the bacteria for the macrophages. This result suggested that something on the bacteria was being recognized by a receptor on the macrophage.

To show that complement was involved in this process, equal quantities of the three strains of bacteria were allowed to activate complement proteins. The types of proteins activated indicated that the polysaccharide activated complement directly, rather than because of prior antigen-antibody reaction.

Furthermore, the three strains of bacteria activated the complement protein C3 in the same way they were phagocytized: the most virulent activated the least C3, the intermediate activated an intermediate amount, and the least virulent activated the most C3.

This mechanism also explains what happens during infection. When the least virulent organism was injected into mice it was removed from the blood by phagocytic cells much more rapidly than the most virulent organism. When the mice were treated with a cobra venom factor known to destroy complement, the rapid removal of the least virulent organism was slowed, while the already-slowed removal of the most virulent organism was unaffected.

These results show that very slight differences in the polysaccharide structure drastically affect the ability to activate complement. These investigations explain an important, previously unrecognized, mechanism of disease causation. Understanding these mechanisms may suggest ways to enhance phagocytosis of pathogenic organisms. ■

Dr. Loretta Leive and her coworkers at NIADDK worked closely with scientists in Japan and Finland to find out why changes in bacteria's cell surface components can make the microbes more or less virulent. NIH PHOTO 1793

Cancer and Occupation: NCI Finds High Risk of Cancer Among Petroleum Refinery Workers

Linda Anderson

Scientists at the National Cancer Institute (NCI) and the National Institute for Occupational Safety and Health (NIOSH) have reported preliminary findings that employment in the oil refinery industry may increase the risk of brain and stomach cancers, and leukemia.

In preliminary results reported in 1980, the scientists found more than the expected number of deaths from brain tumors among the active and retired white male workers of three plants in the Beaumont/Port Arthur area of Texas. The plants' operations consists primarily of standard excess of brain tumor deaths was more pronounced among the active workers than the retired workers. Thirty-three deaths due to brain tumors occurred, compared with 16 that would be expected based on the proportion of brain tumor deaths that occur in the general population of the United States.

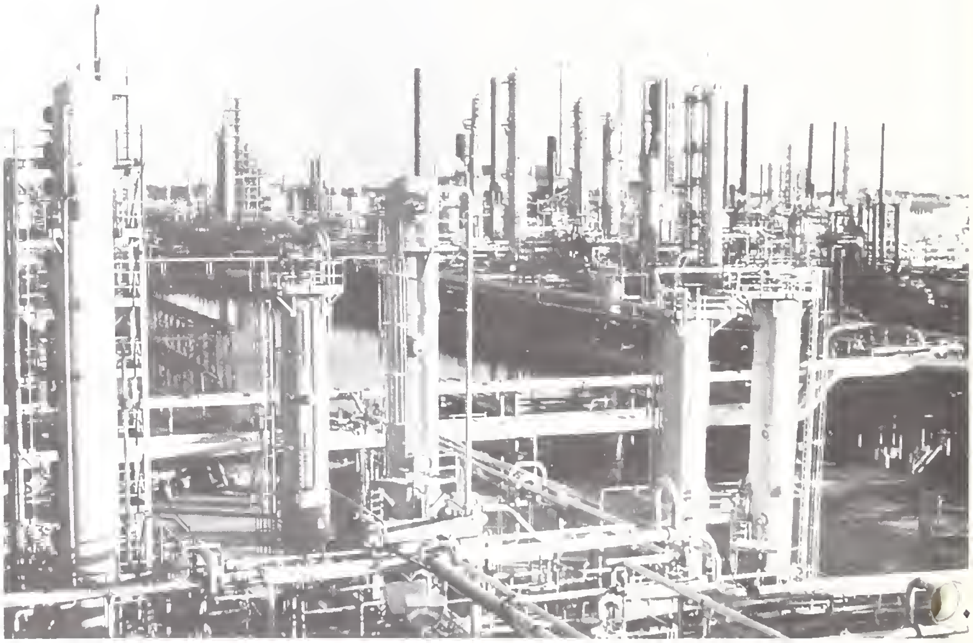
The brain tumors included 27 cancers and six growths that were benign or not specified by type on death certificates. The expected number of deaths would be 12 brain cancers and four benign or unspecified brain tumors.

The scientists examined 2,509 deaths that occurred between 1943 and 1979 among men employed at the three petroleum refineries. They used a statistical method called Proportional Mortality Ratio (PMR) to compare the number of deaths for each type of cancer that occurred in the study group with a calculated expected number, based on the experience of the general population of the United States and the two-country area where the three plants are located. Results of this type of study are considered preliminary because the statistical method used generates clues for further analysis rather than providing definitive estimates of cancer risk.

Studying other cancer deaths, they identified 36 deaths from stomach cancer among the white male workers, compared with 25 that would be expected in the general population. Twelve stomach cancers were observed among nonwhite males while only six were expected. An excess was found among both active and retired workers at two of the plants, and among active workers only at the third.

Thirty-one deaths among the white male workers were due to leukemia, compared with the 16 expected. The elevated frequency of leukemia deaths was more pronounced among the retired workers. There also was a slight excess of deaths from multiple myeloma (a cancer involv-

NIH PHOTO 1794



NIH PHOTO 1795

ing plasma cells of the bone marrow), nine deaths were observed while four were expected.

The increased frequency of cancer deaths identified in this study is consistent with other studies on workplace exposures in the petroleum and petrochemical industries.

The scientists also noted slight excesses of deaths from other cancer types, including pancreatic, prostate and skin cancers among white males and skin cancer among nonwhite males.

At the present time, these findings are considered intriguing, but inconclusive. Consequently, further evaluation of the cancer experience of these workers is now under way. The number of deaths from any specific cancer among OCAW members in these plants is small, making statistical analysis difficult; however, workers' records are being examined in detail for clues to a common workplace exposure that may be responsible for the increased frequencies of certain cancers.

The finding appeared in a recent issue of the *Journal of Occupational Medicine*. The authors are Terry L. Thomas, M.S...and Joseph F. Fraumeni, Jr., M.D., of the NCI Environmental Epidemiology Branch; and Richard J. Waxweiler, Ph.D, of the NIOSH Division of Surveillance, Hazard Evaluations, and Field Studies in Cincinnati in collaboration with Rafael Moure-Eraso, M.S., and Sharon Itaya, M.D., of the Health and Safety Department, Oil, Chemical, and Atomic Workers International Union (OCAW). ■

NCI Studies Two Families for Clues about Risk Factors

Florence S. Karlsberg

Scientists at the National Cancer Institute (NCI) have studied two families with an unusually high number of cases of cancer of the lung and larynx, suggesting a familiar predisposition to such cancers. In both families, smoking and, to a lesser extent, occupation, were evident as risk factors for lung cancer.

The study is aimed at determining whether some smokers are more susceptible than others to the development of lung cancer due to smoking. In family A, from North Carolina, ten in one generation survived to adulthood: five developed lung cancer and one developed concer of the larynx. All six were cigarette smokers who were diagnosed between the ages of 53 and 67. Four of the five men worked as carpenters in the family business. The female patient ran a cafe with her husband. None of the wives or husbands developed lung cancer.

A frequent characteristic of hereditary tumors is an early age at diagnosis. In this family, the mean age at diagnosis was 60.5 years, typical for the general population. However, the simultaneous occurrence in this family of a newly-recognized syndrome (group of symptoms that occur together and characterize a disease) of limb and dental birth defects may be related to their risk of developing cancer. An association between certain birth defects and childhood neoplasms was reported in 1964, by Robert W. Miller, M.D., chief of the NCI Clinical Epidemiology Branch. A variety of familial and genetic syndromes is now linked to cancer susceptibility.

Two of the lung cancer patients, a brother and sister, were born with one arm shorter than the other. Six offspring of this sister and another brother, who had neither lung cancer nor birth defects, were born with deformities of limbs, teeth, or both; a seventh was born with Down's syndrome. Two of the members with limb defects also had lung cancer. The five cancer-free individuals with birth defects are less than 40 years of age and may yet develop lung cancer.

Such limb and dental defects may be due to faulty development of the embryonic structure from which the nervous system is derived. Known as the neural crest, it contributes to dental development and it is believed that limb defects may result from interference with its normal development. Oat cell carcinoma of the lung cancer may also be due to faulty development of the neural crest and was recently found by other NCI scientists to have a characteristic chromosomal abnormality. Family A's predilection for lung cancer and limb and dental anomalies may be partially explained as a defect of neural crest development.

Family B came from northern Virginia. Among eight children of a mother with cancer of the jaw and a father with no reported cancer, four developed lung cancer and one developed cancer of the larynz. All smokers, these five siblings were diagnosed between the ages of 49 and 79. All had been raised on a farm with possible but unverified exposure to pesticides. As adults, they worked as clerks, slesmen, and housewives, with no exposure to known occupational hazards. Among their 11 children, lung cancer occurrred in three family members older than 40.

To prevent further deaths from cancer of the lung and larynx in families A and B, counseling measures were started. Family members received routine blood and chemistry studies, were informed about early cancer symptoms, were encouraged to avoid smoking and occupational hazards and to have regular chest X-rays and sputum analyses.

Tests were done on six family A and four family B members to see if the predisposition for cancer and birth defects was related to the same chromosomal defect. Abnormal chromosome patterns (balanced 13-14 translocation) appeared for two family A members. Since neither had birth defects and only one had lung cancer, and no other family members with lung cancer were available, no conclusions could be drawn about the role of the abnormal chromosone.

In the hope that tests may be developed that will serve as markers of risk for lung cancer, blood and tissue specimens have been stored for future use.

These family studies were conducted in the Clinical Epidemiology Branch, NCI, by Thomas E. Goffman, M.D., Dawn D. Hassinger, M.D., Ph.D., and John J. Mulvihill, M.D. and reported recently in *The Journal of the American Medical Association.* ■

NIH Studies Benefits of Aspirin and Vitamin A precursor in Preventing Heart Disease and Cancer

Bill Sanders

The National Heart, Lung, and Blood Institute (NHLBI) and the National Cancer Institute (NCI) are jointly supporting a five-year clinical trial to ascertain whether aspirin and beta-carotene are useful in the primary prevention of cardiovascular disease and cancer.

Approximately 22,000 subjects between the ages of 40-75, all of them physicians, are being invited to participate. Each participant will take one pill daily: on even days, it will be either 325 mg. of aspirin or a placebo; on odd days, it will be either 30 mg. of beta-carotene or a placebo. For convenience, the pills will be supplied in calendar packs.

Every six months, each participant will be sent a new supply of pills together with a follow-up questionnaire on which he will report his compliance with the regime and the current status of his health. At that time he will also provide signed permission for the investigators to examine medical and hospital records relating to any illness reported.

Aspirin inhibits the aggregation, or "clumping" of blood platelets, an early and perhaps essential step in the development of blood clots. Clots and emboli are often involved in or directly responsible for many of the disabling or lethal manifestations of heart and blood vessel diseases—including a high proportion of heart attacks and strokes. Aspirin and other "antiplatelet" drugs have been considered a promising approach to primary and secondary prevention of such thromboembolic complications among persons at high risk due to arteriosclerosis or other cardiovascular conditions.

As a measure for stroke prevention, aspirin has performed well in several clinical trials, significantly reducing the incidence of transient ischemic attacks, completed strokes, and cerebrovascular-disease deaths among high-risk males (but, surprisingly, not among high-risk women).

As a means of secondary prevention among men who had previously sustained one or more heart attacks, aspirin has been generally disappointing. The NHLBI-supported Aspirin-Mycardial Infarction Study disclosed no significant benefits from aspirin therapy. The drug did slightly reduce the threat of recurrent heart attacks, but had no effect on total or cardiovascular-disease death rates.

However, another study, evaluating aspirin or aspirin plus dipyridamole, suggested that aspirin or combination therapy did have a favorable (though not striking) effect on the incidence of recurrent heart attacks and on mortality, especially if therapy was initiated early after the acute episode.

The results of clinical trials to date slightly favor the use of aspirin during the early months after a heart attack, though the evidence for its benefits remains inconclusive. More research is needed as to the value in the primary prevention of thromboembolic episodes.

Another unresolved question is whether low doses of aspirin might be more effective in the prevention of thromboembolic complications than higher doses. The drug is thought to inhibit the aggregation of platelets by blocking their production of thromboxane A^2, a blood vessel constrictor and aggregating agent. It appears to do this effectively at doses as low as half a tablet every other day.

Most clinical trials of aspirin have employed doses of a gram or more daily. At these dosages, aspirin may produce significant side effects in some subjects; but, perhaps more important, it may impede production in the blood vessel wall of prostacyclin.

Prostacyclin, through derived from the same precursor substance (arachidonic acid) as is thromboxane A and involving a common enzyme (cyclo-oxygenase) in its synthesis, produces diametrically opposite effects: it dilates blood vessels and inhibits platelet aggregation. By inhibiting the common enzyme, cyclo-oxygenase, aspirin can curtail production of both thromboxane A^2 (a desirable effect) and prostacyclin, thought to be protective against intravascular clotting.

The present study will be the first large-scale clinical test of low-dose aspirin in the primary prevention of thromboembolism to be conducted in the United States. A smaller trial is already underway in Great Britain.

The addition of beta-carotene to the study regimen will provide information on the prevention of tumors. Beta-carotene is a precursor of vitamin A, which has been shown in animal studies to protect against the induction of tumors by various techniques. But, the doses of vitamin A needed for significant antitumor activity could pose serious toxicity problems in humans.

However, some dietary studies in humans have indicated that a diminished cancer risk was more strongly associated with the beta-carotene content of the subjects' diets than with their vitamin A content. Moreover, there is no evidence that the high physiological levels of beta-carotene to be used in this study are associated with any serious side effects, though some slight yellowing of the skin may occasionally occur.

The study will be carried out by Dr. Charles H. Hennekens and colleagues at Harvard Medical School. ■

The following papers were presented at the Annual Federation of American Societies for Experimental Biology (FASEB) Meeting held in New Orleans in April.

Scientists Discover Transforming Growth Factors that May Detect Cancer Earlier

Malignant cells appear to produce transforming growth factor in cancer patients as well as in cell cultures, an observation which could be useful for early cancer detection.

National Cancer Institute (NCI) researchers have reported finding transforming growth factor in the urine of 18 out of 22 cancer patients tested. Daniel R. Twardzik, Ph.D., Stephen A. Sherwin, M.D., and George J. Todaro, M.D. suggest that assays for this growth factor might help physicians detect cancer before it is otherwise apparent and monitor the course of cancer therapy.

Recently, scientists have discovered that human cancer cells grown in artificial media produce various growth-sustaining substances. Some of these are small proteins, called "growth factors," that stimulate both normal and tumor cells to divide. Others, termed "transforming growth factors," appear capable of stimulating tumor cells to grow as colonies in artificial media containing a small amount of agar. Normal cells cannot grow in agar-containing media, but cancer cells can.

Just because growth factors are produced by cultured cancer cells does not mean they are produced by tumors inside people. To find out if they are, the NCI investigators collected urine samples from patients with cancer of the lung, breast, colon, ovary, pigment cells, or fibrous tissue, from patients with other diseases, and from healthy individuals. The biochemical properties of transforming growth factors, the investigators explained, indicate that they would be stable and therefore detectable in urine.

All of the urine specimens were subjected to biochemical procedures in order to isolate that portion of the urine containing growth factors. Urines were acidified, centrifuged to remove debris, and separated by a chromatography system that allows molecules of different sizes to be studied independently. Portions of the processed urine were then tested for their ability to stimulate the growth of cell colonies in agar.

The researchers found transforming growth factor in the urine of 18 of 22 cancer patients tested. Only five out of 25 urine specimens from healthy people contained transforming growth factor. The particular factor isolated appeared to be a relatively small protein which, the cancer investigators noted, might have been missed in some urine samples if it was present in only very small concentrations.

The NCI team suggested that in the future transforming growth factor might prove a useful "tumor marker" for the early detection of cancer and to monitor the progress of cancer therapy. Other tumor markers have been used successfully for this purpose in the past. But these markers, including carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP) probably have nothing to do with the cancer's unique biological activity. The activity defined by transforming growth factor, the ability to allow cell growth under ordinarily unfavorable conditions, may have something to do with what makes a cell malignant.

In future studies, the NCI group will try to correlate the activity of transforming growth factor in the urine of cancer patients with the patient's response to treatment and with the patient's tumor size. The investigators hope to develop animal models for the study of transforming growth factor. The animal models may allow the cancer researchers to develop new therapeutic approaches which control the expression of transforming growth factor, thereby inhibiting tumor cell growth. ■

Human Antibody Inhibits Tumors in Mice

An antibody to human colorectal cancer has been found to effectively inhibit gastrointestinal tumor growth in hairless mice, according to research supported by the NCI.

Dorothee M. Herlyn, DVM, and Hilary Koprowski, MD, of The Wistar Institute in Philadelphia have found that an anti-colon cancer antibody inhibited tumor growth even when it was used seven days after cancer cells were inoculated. Initial clinical studies on seven cancer patients showed that the antibody produced no ill effects.

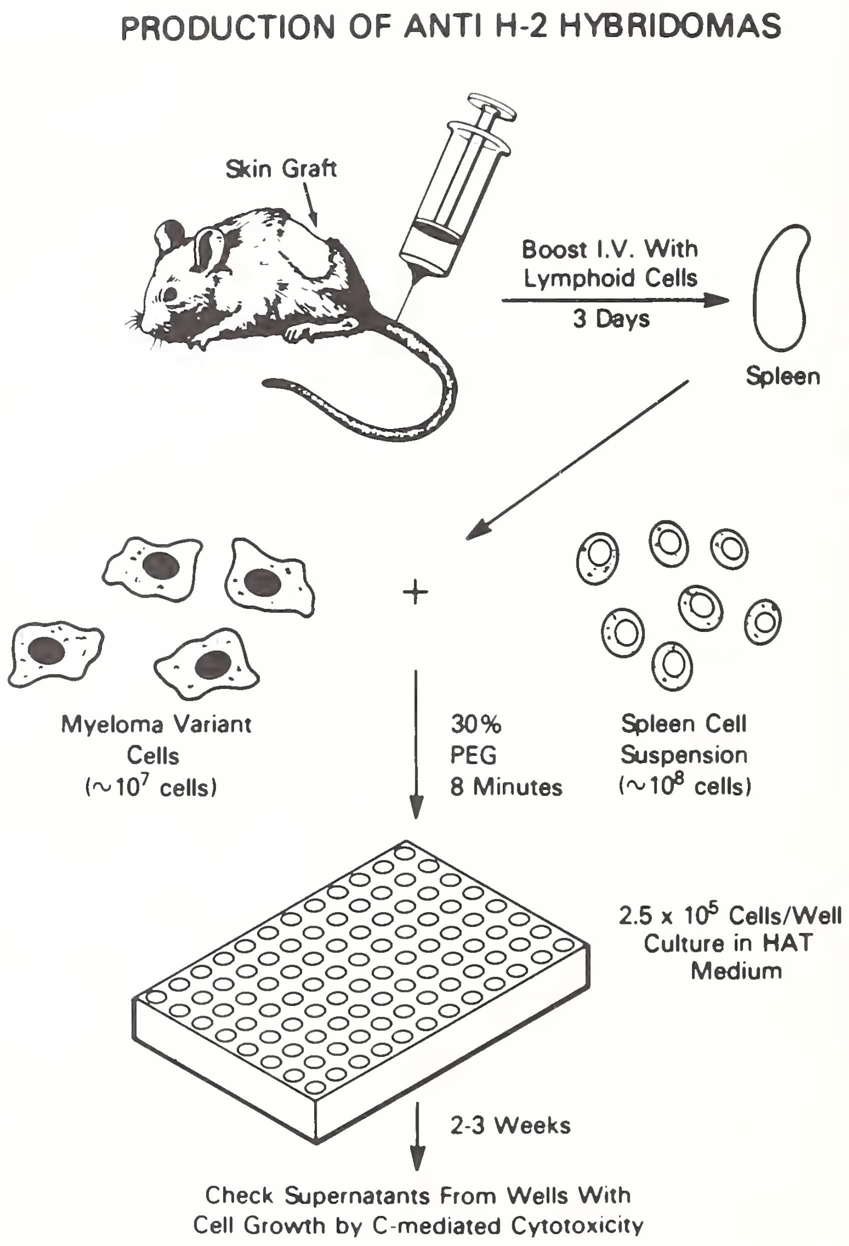
Antibodies to tumor antigens (proteins on cancer cell surfaces which react with specific antibodies) have been traditionally produced by injecting animals with cancer cells and then collecting antibody-containing blood sera. Attempts to use immune sera from animals for human cancer therapy have been limited, partly because of the difficulty in obtainng sera that react only with tumor antigens and not normal antigens. Furthermore, it is extremely difficult to produce these sera in sufficient quantity for use in treating people.

Recent technical advances in somatic cell hybridization have changed the immunotherapy picture. In this procedure, two different cell types are fused in such a way that desired functions of both cell types are saved and expressed by the hybrid cell. The Wistar Institute investigators have used somatic cell hybridization, fusing mouse lymphocytes (white blood cells) which produce antibodies against human tumor cells with mouse blood cell tumors. More than 200 different antibodies against cancer of the gastrointestinal tract, skin cancer, and lung cancer have been established. Five of these antibodies, the investigators have found, inhibited tumor growth in nude mice, hairless animals which, because of a defect in the immune system, support the growth of human tumors. All of these effective antibodies, the investigators noted, belong to the same immunoglobin class.

Drs. Herlyn and Koprowski noted that in a culture, anti-colon cancer antibody binds only to human tumors of the gastrointestinal tract. It ignores normal cells and other tumor cells. One single injection of the antibody given immediately after tumor cell inoculation, or even seven days later, effectively inhibits tumor growth in nude mice. The growth-inhibiting properties of this antibody seems to be very specific, making it a good candidate for the treatment of human colorectal cancer.

Further experiments with the anti-colon cancer antibody revealed that it probably destroys tumor cells in mice by working in conjunction with macrophages, large immune cells that engulf and destroy foreign cells and particles in the body.

Drs. Herlyn and Koprowski suggest that their antibody has immunotherapeutic possibilities for human colorectal cancer. Initial clinical trials have shown no adverse side effects from the antibody which could be detected in the patients' blood sera for up to two weeks following a single injection. If further trials prove as successful, this antibody may one day help inhibit colorectal cancer growth in man.■


PRODUCTION OF ANTI H-2 HYBRIDOMAS
Skin Graft
Boost I.V. With Lymphoid Cells
3 Days
Spleen
+
Myeloma Variant Cells ($\sim 10^7$ cells)
30% PEG 8 Minutes
Spleen Cell Suspension ($\sim 10^8$ cells)
2.5 x 10^5 Cells/Well Culture in HAT Medium
2-3 Weeks
Check Supernatants From Wells With Cell Growth by C-mediated Cytotoxicity

NIH PHOTO 17

Scientists at the Wistar Institute in Philadelphia have found an antibody to colorectal cancer that can successfully inhibit gastrointestinal tumor growth in hairless mice. Research procedures include fusing mouse lymphocytes, which produce antibodies against human tumor cells with mouse blood cell tumors. Five antibodies were found to inhibit tumor growth in the laboratory animals, and all five of these antibodies belong to the same immunoglobin class.

Aspirin Shrinks Liver Tumors in Rats without Side Effects

Aspirin inhibits the growth of liver tumors in laboratory rats without the toxic side effects usually seen in cancer chemotherapy, according to NIH grantees at Howard University.

Enid M. Knight, Ph.D., and fellow researchers E. Marsh, A. Stoddart, J. Fakunle, and W.E. Criss at Howard, hope that their research will help answer some questions about diet, chemotherapy and the side effects of the common over-the-counter drugs against cancer.

Most clinicians and researchers use a wide array of drugs to inhibit tumor growth in man and laboratory animals. But the drugs cause a number of side effects that are mildly unpleasant and sometimes toxic. In many cases, physicians must decide which is worse for a patient—the cancer or the treatment.

Loss of appetite and poor nutrition are fairly common side effects of chemotherapeutic agents. Many investigators believe that poor nutrition can reduce a cancer patient's chance of recovery. With this in mind, the Howard University researchers have begun to study the side effects of diet and some common drugs on tumor growth in experimental animals.

In their recent study, which was supported in part by grants from the National Cancer Institute (NCI) the Howard investigators looked at the tumor-inhibiting effect of two common over-the-counter pain relievers—salicylic acid (aspirin) and salicylamide (an aspirin derivative)—in buffalo rats. These two drugs were used because they are relatively nontoxic.

To maintain adequate nutritional status, the animals were fed a high protein diet for two weeks before tumor transplantation and throughout the rest of the experimental period. Aspirin or aspirin derivative was given to two groups of animals along with the high protein diet for three days before tumor transplantation. A third group of animals was given no drugs. All of the animals were injected with about a million liver tumor cells.

The growth of the animals, tumor area, and symptoms of toxicity were recorded at intervals during the following four weeks. After the experimental period, tumors and blood sera were analyzed for tumor protein, nucleic acids, tumor fat, and serum proteins.

The Howard team found that the rats on the aspirin diet were more alert and energetic than the other animals. At times the aspirin-fed rats exhibited symptoms of hyperactivity. The latency period for tumors—the time that elapsed between tumor cell injection and the appearance of detectable tumors—was significantly longer in the aspirin-treated rats than in drug-free or salicylamide-fed animals.

Two weeks after tumor cell injection, none of the animals in the aspirin diet had measurable tumors while tumor measurements were recorded for all the other rats. Once tumors appeared in the aspirin-fed animals, they were significantly smaller than those measured in the other rats.

Dr. Knight and his fellow workers concluded that aspirin has a more potent inhibitory effect on the growth of transplanted liver tumors than either salicylamide fed with a protein rich diet or a high protein diet alone. Furthermore, aspirin works without apparent toxicity.

Dr. Knight was careful to add that these studies are preliminary and provide information on one type of tumor in one animal species. Further research with diet and aspirin or other over-the-counter drugs is still needed.■

Rise in Temperature after Activity may Explain Why Exercise Can Prevent Infection

The same protein that produces fever during infection may be partly responsible for elevating body temperature during exercise, a possibility which, if proven true, might explain the claim that regular exercise wards off infection.

University of Michigan physiologists Joseph G. Cannon, MS, and Matthew J. Kluger, PhD., supported by the National Institute of General Medical Sciences have found that plasma samples taken from research participants after one hour of exercise were able to increase body temperatures and decrease blood iron concentrations in laboratory rats. These same effects, the investigators noted, are produced by endogenous pyrogen, the protein responsible for fever during infection.

The temperature elevation accompanying physical activity, such as running, or doing household chores, is a common experience to which we pay little attention. Many physiologists are interested in temperature elevation and have been trying to figure out how and why it works.

Some researchers have suggested that the elevated body temperature in exercise, like the fever during infection, represents a resetting of the body's "thermostat." Studies have shown that fever is probably mediated by a protein released from white blood cells, and stimulates the synthesis of certain plasma proteins. These "acute phase" actions of endogenous pyrogen during infection create an environment within the body unfavorable to bacterial growth and multiplication.

The Michigan investigators wanted to find out if endogenous pyrogen concentrations increase during exercise. They know that body temperature increases during exercise, and that the number of circulating white blood cells also increases.

The researchers tested their idea with the help of volunteers and laboratory rats. Plasma samples were taken from research volunteers after one hour of submaximal exercise on bicycle exercise machines and injected into the animals. The rats responded with increased rectal temperatures and decreased plasma iron concentrations. Injections of plasma taken from the same people before exercise had no effect on the rats.

The Michigan physiologists cannot say for certain that the temperature-elevating substance they found in the plasma of exercisers is indeed endogenous pyrogen. Further studies are planned to look for changes in the concentrations of the plasma proteins, fibrinogen, haptoglobin, and C-reactive protein in rats injected with plasma from human exercisers. If these concentrations increase, the argument which favors endogenous pyrogen as the temperature-elevating protein in exercise will be even stronger.

The question still remains—is hyperthermia during exercise a fever? Not enough evidence has been gathered yet to answer that question. But if the answer is "yes," it could explain why body temperature often remains elevated for several hours after exercise stops. And it suggests that regular exercise, by inducing physiological changes hostile to bacterial growth, may help prevent infection.

Pregnant Rats that Smoke Help Scientists Learn about Effects on Fetus

An automatic "smoking" machine and laboratory rats should help scientists learn more about what happens to fetuses when their mothers smoke, report Columbia University investigators Pedro Rosso, M.D., Jorge A. Bassi, M.D., Adrien Moessinger, M.D., and L. Stanley James, M.D. The NCI grantees use an exposure machine to set up a reproducible model of maternal smoking during pregnancy in the rat.

Preliminary experiments with the model have revealed that not all parts of the fetus' body are affected equally by smoking. The lungs appear to be more affected than other organs, showing a decrease in both cell number and size.

Low birth weight is associated with a host of developmental problems that affect a child well past infancy. Among the many factors that have been linked to low birth weight is maternal smoking during pregnancy. Babies born to women who smoke during pregnancy are, on the average, seven ounces lighter than babies born to nonsmokers.

The evidence supporting a causal relationship between maternal smoking and reduced birth weight is fairly substantial. The results have been consistent in all the studies performed regardless of country, race, culture, or geographic setting.

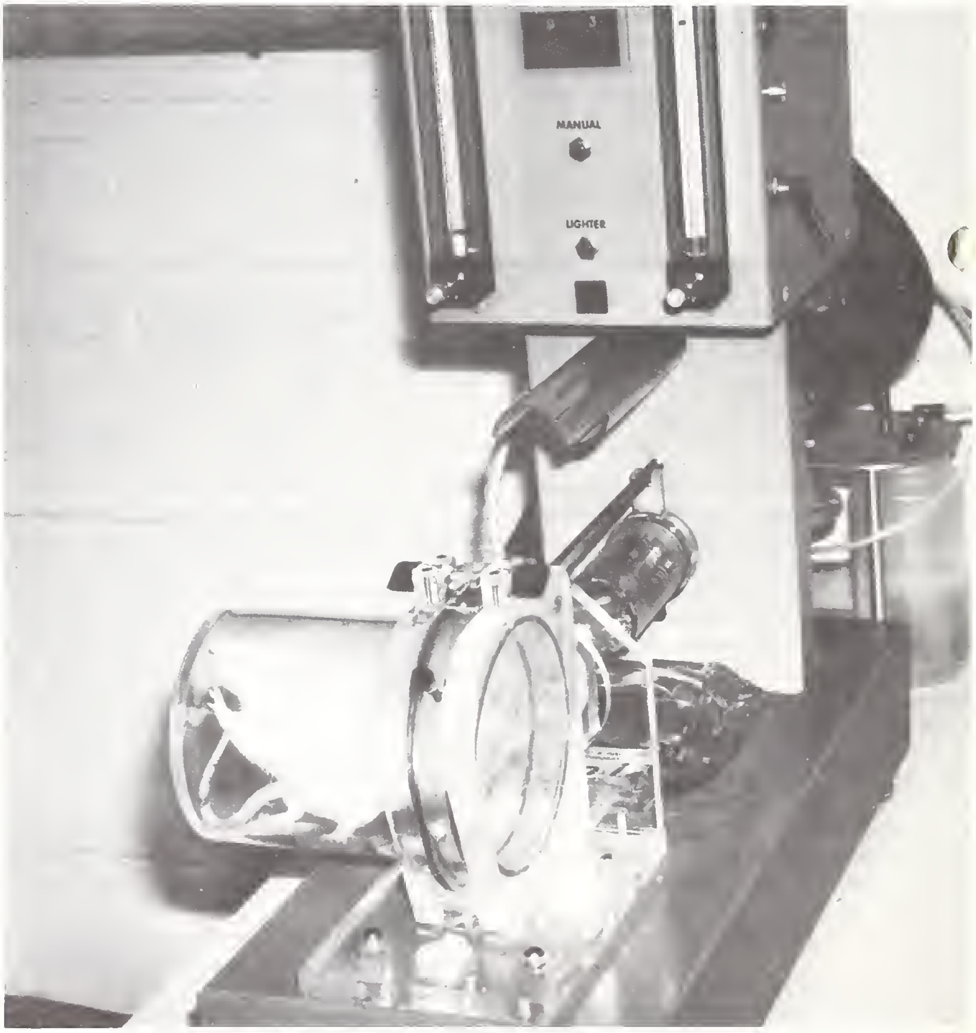
Furthermore, the relationship between smoking and reduced birth rate is independent of all factors. These factors include race, the mother's size, sex of the child, socioeconomic status of the mother, and the number of children the mother has already given birth to. Also data reflects the more a woman smokes during pregnancy, the smaller her baby will be.

Dr. Bassi and his co-workers have been using a rat model to find out what happens to fetuses when their mothers smoke. The pregnant rats are given free access to food and water and exposed to tobacco smoke. Smoke exposure is accomplished by placing the rats in a chamber attached to a "smoking" machine. The machine delivers two-second air puffs through three lighted cigarettes. The smoke from the cigarettes enters the exposure chamber. In a typical one-minute cycle of operation, the two-second puff is followed by a 28-second hold period, for a total smoke exposure time of 30 seconds. This is followed by a 30-second purge period in which the smoke is swept out of the chamber. The cycle is repeated until a cigarette is consumed, a process that takes from six to eight minutes. Using this procedure, the Columbia team exposed the pregnant rats to smoke from a cigarette every 30 minutes, from 9 AM to 5 PM daily, from the 5th to the 20th day of gestation.

On the 21st day of gestation, the investigators examined the rat fetuses for gross weight and malformations and for abnormalities in various body tissues. The fetuses of smoke-exposed animals were compared to normal rat fetuses and to fetuses carried by rats on restricted diets.

The researchers found a significant growth retardation in fetuses of smoke-exposed rats. Tobacco smoke is apparently even more harmful to fetal growth than food restriction. The growth retardation, the investigators noted, involved a decrease in the weight of the whole fetus, in the number of liver cells, and in the number and size of lung cells.

Dr. Bassi concludes that by using an automatic smoke exposure machine, he and his colleagues have established a standardized animal model which should aid future studies.



NIH PHOTO 1

The "smoking machine" contains a chamber that houses the pregnant rat. The machine delivers two-second air puffs through three lighted cigarettes. The researchers found a significant growth retardation in fetuses of smoke-exposed rats.

Photo compliments of Dr. Bassi, Columbia University.

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Ms. Maureen Napper
National Institutes of Health
Building 31, Rm. 2B-10
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National Institutes of Health

NIMH Researchers Reveal Their "Secret" **p. 9**

New Diagnostic Tool Detects Heart Disease Before Symptoms **p. 14**

New Hope for Babies with Blood Vessel Defect **p. 11**

Cause of Lyme Disease May Be Bacteria **p. 3**

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

NIH Photo 1798

News&Features from NIH

June/July 1982

Two Chinese neuroscientists at NIH used chemical blockers to prolong the pain-killing effects of acupuncture. Their research adds to evidence that acupuncture triggers the brain to release endorphins, (opiate-like substances) impact explaining the effects of this Chinese treatment. See page 9

Multiple sclerosis victims have a breakdown in myelin-associated glycoprotein, according to NIH researchers. In multiple sclerosis, myelin is destroyed and nerve impulses are slowed or blocked leading to muscle weakness and abnormal movements. The reduction in glycoprotein found in MS suffers may help scientists detect and possibly treat the disease. See page 2

Hyperactive children receive special attention in the hyperactivity program at the NIH clinical center. As they participate in games and therapy, researchers are learning valuable information about the disorder. See page 10

NIH Photo 1799

NIH scientists have found abnormalities in the immune system of homosexual men who use inhalants such as amyl and butyl nitrite as sexual stimulants. The recent outbreak among these men of Kaposi's sarcoma—a rare skin cancer—and opportunistic infections may be linked to these abnormalities. See page 13

Phenobarbital—an hypnotic sedative—can produce permanent damage to the reproductive systems of animals exposed to the drug in the womb. Tranquilizers and sedatives represent the major group of drugs prescribed to pregnant women. Although the clinical significance of this finding is unknown, one thing is certain, phenobarbital should be prescribed cautiously to pregnant women. See page 7

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Editorial Staff

Mary Donovan
Editor
Susan Gerhold
Associate Editor
Helen Ou
Special Assistant
Maureen Napper
Editorial Assistant

NIH News Branch
Building 31, Room 2B-10
Bethesda, Maryland 20205
Phone:
(301) 496-2535

Glycoprotein Degradation May Provide Clues to Understanding Multiple Sclerosis

Ray Fleming

The breakdown of a recently identified glycoprotein found in layers of nerve-protecting myelin could be an important clue to understanding multiple sclerosis (MS), says the scientist whose research team has discovered and investigated the phenomenon.

According to Dr. Roscoe O. Brady, chief of the Development and Metabolic Neurology Branch at the National Institute of Neurological and Communicative Disorders and Stroke, a reduction in myelin-associated glycoprotein (MAG) occurs in the inner layers of fatty myelin when multiple sclerosis attacks the body. Dr. Brady and his NINCDS colleagues Drs. Richard H. Quarles and Henry Webster have also found that MAG disappers in normal-appearing tissue surrounding MS lesions in the brain—the first change detectable in the process of demyelination.

In multiple sclerosis, demyelination is characterized by the destruction of patches of nerve-insulating myelin—damage that blocks or slows down nerve impulses traveling through the brain and spinal cord. Demyelination, though frequently associated with MS, is also a symptom of other neurological disorders. Dr. Brady believes that the discovery of the destructive process in MS could help patients with the other disorders in which myelin may be affected.

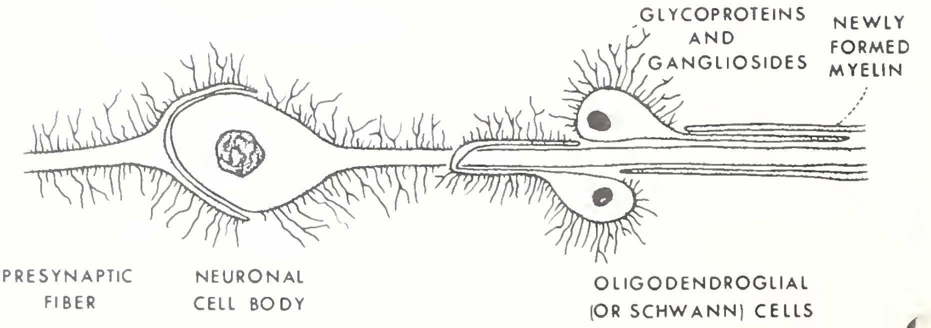
In the early 1970's, Drs. Quarles and Brady began to look at the biochemical mechanisms of myelin development to learn more about what may cause myelin to disintegrate. Their theory of myelination, now generally accepted, was that during cell migration in early life, certain cells (called oligodendroglial cells) in the central nervous system interact with the axons of nerve cells. Specific glycoproteins (proteins with a carbohydrate group attached) in the membranes of the oligodendroglial cells, and other glycoproteins on the surface of axons, cause the cells to be drawn tightly to the axons. Once axon and oligodendroglial cell are next to each other, the oligodendroglial cell produces myelin in a layered sheath around the axon.

MAG—one of the glycoproteins in the membrane of the oligodendroglial cell—has now been isolated and chemically characterized. It is selectively located on the inside of the myelin sheath next to the axon—where myelin formation begins—suggesting that MAG is involved in the interactions between the oligodendroglial cell and the axon.

Drs. Brady and Quarles adjust radioimmunoassay equipment used in MAG detection studies.

NIH Photo 1800

MYELINATION
A THEORETICAL SCHEME


GLYCOPROTEINS AND GANGLIOSIDES
NEWLY FORMED MYELIN
PRESYNAPTIC FIBER
NEURONAL CELL BODY
OLIGODENDROGLIAL (OR SCHWANN) CELLS

The diagram shows the MAG—myelin-associated glycoprotein—located on the insde of the myelin sheath next to the axon, where myelin formation begins. When multiple sclerosis attacks the body, a reduction in MAG occurs in the inner layers of the fatty myelin.

NIH Photo 1801

Although myelin is formed from glycoprotein-rich cell membranes, the maturing layers were thought to contain little or no glycoprotein. Drs. Quarles and Brady, however, found MAG in myelin isolated from the rat and later from the human central nervous system. They also found that as successive myelin layers matured and became more closely bound together, the size of MAG was reduced by as much as 10 percent. These discoveries led the scientists to speculate that MAG was produced to promote formation of new myelin and to control the maturation process in which myelin layers are bound together.

Intrigued by this hypothesis, Drs. Brady and Quarles were joined by Dr. Webster and other scientists in studying the distribution of MAG in myelin damaged by multiple sclerosis. The results were striking. The scientists, using immunohistochemical and radioimmunoassay techniques, found evidence of the selective losses of MAG in MS-damaged myelin. Even more importantly, normal-appearing tissue in the area of MS lesions showed evidence of considerable MAG breakdown. This breakdown, which was found to occur before myelin destruction, was the first biochemical or structural change detectable in the process of demyelination.

How this early MAG breakdown relates to the cause of multiple sclerosis is a source of some speculation. Since membrane glycoproteins like MAG are known to be both receptors for some viruses and cell-surface antigens, MAG may play a key role in a viral or in an autoimmune reaction in this disease. It is possible that a virus could alter the immune reactivity of MAG, so that it becomes a foreign antigen and subject to attack by the MS patient's own immune system. One aspect of current research is to determine if the immune system of MS patients has been activated to react with MAG. In a related investigation, evidence has been obtained that an enzyme which affects proteins, called a protease, is altering MAG, and that the enzyme's activity is higher in MS tissue than in the normal brain.

Much research remains to be done before the full impact of Drs. Quarles and [illegible]dy's original work is known for the demyelinating disorders. "Obviously, we're hoping for an abundant harvest," says Dr. Brady. So far, the fields they have plowed have been fertile indeed. ■

Probable Cause of Lyme Disease Isolated

Patricia Randall

Staff scientists of the National Institute of Allergy and Infectious Disease's Rocky Mountain Laboratories (RML) have detected in and isolated from ticks a spirochete that may be the causative organism of Lyme disease (sometimes called Lyme arthritis). A spirochete is a spiral-shaped form of bacterium.

Since the disease first was observed in Lyme, Connecticut in 1975, epidemiologic evidence has pointed to ticks of the genus Ixodes as the vector (transmitting agent), but no disease organism has been identified.

Lyme disease usually begins with a skin lesion called erythema chronicum migrans (ECM). Weeks to months after these lesions appear, neurologic or cardiac abnormalities and various forms of arthritis may follow. Recurrences of the inflammation and joint pains are intermittent and have not been tied to a specific cause. Penicillin therapy shortens the duration of ECM and may prevent or attenuate the subsequent arthritis.

The ticks, *I. dammini,* were collected from vegetation on Shelter Island, NY, a known focus of Lyme disease. Investigators at RML detected spirochetes in 61 percent of the ticks collected, varying from a few microorganisms in some ticks to large clumps in others. The spirochete recovered from the ticks now has been grown in pure culture in the laboratory. Electron microscopy revealed that it is similar in structure to the *treponemes,* one species of which causes syphilis.

In tests with New Zealand white rabbits, the scientists found that EMC-like lesions developed in the skin of rabbits fed upon by infected ticks. Sera of all the rabbits fed upon by such ticks contained antibodies to the specific spirochete 30 and 60 days after tick exposure.

The investigators also demonstrated that the spirochete binds antibodies in sera of patients recovering from Lyme disease. They studies sera from nine patients who had clinically diagnosed Lyme disease, that is, diagnosis had been made based on symptoms, since no laboratory tests have existed. Sera diluted up to 1280 times contained antibodies to the *I. dammini* spirochete, the investigators found. For comparison, they tested sera of 14 persons with no history of Lyme disease. At any level greater than 20 dilutions, there was no reaction with the spirochete.

This, the scientists observed, suggests that the organism "may be involved in the etiology (cause) of Lyme disease." In the first paper published describing their findings, they note that organisms with the structural characteristics of spirochetes were said to be associated with ECM in Europe more than 30 years ago. The association was never confirmed, but the improvement of symptoms of Lyme disease following penicillin therapy also points to a penicillin-susceptible bacterium as a causative agent.

The investigators, staff of the Epidemiology Branch and Laboratory of Microbial Structure and Function of the Institute's Rocky Mountain Laboratories in Hamilton, Montana, suggest that many unanswered questions concerning the significance of the spirochete and its relationship to the *Ixodes* tick now can be answered through experimental epidemiological studies.

Toward that end, the NIAID plans collaborative studies with the New York State Department of Health and with the Wisconsin State Departments of Health and of Fish and Wildlife.

A report of the isolation of the spirochete by NIAID investigators appears in the June 18 issue of *Science.* The authors are Willy Burgdorfer, Ph.D., Head, Arthropod-Borne Diseases Section, Alan G. Barbour, M.D., Laboratory of Microbial Structure and Function, and Stanley F. Hayes, Operation Branch, all of RML: Jorge L. Benach, Ph.D., New York State Department of Health; Edgar Grunwaldt, M.D., Shelter Island, NY; and Jeffrey P. Davis, M.D., Wisconsin State Department of Health and Social Services. ■

New Class of Biological Compounds May Change Treatment of Asthma

Joan Hartman

Identification and characterization of a class of biological compounds promises to change dramatically understanding and treatment of allergic asthma and other hypersensitivity disorders. Current thinking about some inflammatory diseases and coronary artery disease also may change significantly.

The compounds are leukotrienes. Chemically characterized only three years ago, they are 600 to 9000 times as potent as histamine, which causes the itching, sneezing and tissue swelling produced by allergic reactions.

Leukotrienes had been recognized for some time to constrict small airways in the lungs. More recent research indicates they also increase permeability of small blood vessels, constrict coronary arteries and cause white blood cells to concentrate at inflammatory sites as in rheumatoid arthritis. Human studies now have substantiated their role as a cause of breathing difficulties in asthma.

In the April 9 issue of *Science,* Drs. K. Frank Austen and Robert Lewis of the Harvard Medical School reported on comparisons in normal volunteers of reactions to leukotrienes and to histamines. They measured the amount of each substance necessary to cause a 30 percent decrease in forced expiratory lung volume. The investigators, grantees of the National Institute of Allergy and Infectious Diseases, showed leukotrienes to be up to 9000 times more potent than histamines in affecting pulmonary function of the small airways.

These findings, they concluded, "are consistent with a role for leukotrienes as a major mediator (activator) of allergic airway constriction."

Present understanding of leukotrienes is based on accrued findings of only a few research groups worldwide, working independently. Among them are Dr. Austen's group at Harvard and that of another NIAID granteee, Dr. Charles W. Parker of the Washington University School of Medicine.

The mystery began more than 40 years ago with the discovery of slow reacting substance (SRS), a material that caused constriction of smooth muscle in sensitized animal tissue samples. Over the years investigation increasingly linked SRS to compounds released by the body during allergic reactions.

Immediate allergic reactions occur when mast cells and basophils release quantities of histamines and other chemicals. These cells are found in large numbers in the blood and in tissues of the lungs, skin and the linings of the nose and intestinal tract.

As early as 1960, it has been shown that SRS was distinct from histamines. Antihistamines did not inhibit SRS-caused constriction, and the response to SRS developed slowly (thus its name), while response to histamines was immediate.

Technical limitations hampered identification of the source and chemical nature of SRS during the 1960's and early 1970's. Nonetheless, Dr. Austen and others in his laboratory demonstrated the link between the ill-defined SRS and constriction of small peripheral lung airways. This tie to asthma symptoms not suppressed by antihistamines kept interest in SRS alive.

To move the research forward, investigators needed a reliable single source for producing large quantities of SRS. Dr. Parker and his colleague, Dr. Barbara Jakschik, found it in rat basophilic leukemia cells (RBL). Early studies by the Harvard group and by Drs. Lawrence Lichtenstein and Andrew Grant of the John Hopkins University indicated that basophils and mast cells were involved in SRS production; the St. Louis group expanded on this tantalizing lead.

Beginning their pioneering work on the chemistry of these inflammatory mediators in 1975, they contributed much of the definitive information that led to eventual characterization of the structure of SRS. They knew it was an acidic lipid. They knew that another long chain fatty acid, arachidonic acid (AA), played a key role in allergic response and also was found in the membranes of mast cells and basophils.

Drs. Parker and Jakschik incubated RBL cells overnight with AA labeled with radioactive material; separated out SRS produced by the RBL cells; purified the SRS; and then successfully identified radioactive material that had been incorporated into its structure. Thus, they showed that AA was a starting point (precursor) for SRS production.

Other laboratories almost immediately confirmed these findings using different technologies, and investigators turned their attention to identifying which of two known metabolic pathways of AA synthesized SRS.

One pathway results in the production of prostaglandins, a family of compounds that regulate both smooth muscle contraction and dilation. The prostaglandins, along with histamine, can constrict the large central passages of the lungs responsible for asthmatic episodes.

Through an elaborate series of experiments, Dr. Parker's group showed that blocking this pathway actually increased SRS production. This confirmed that it is the second pathway that generates SRS molecules. Again these findings were confirmed almost simultaneously by the independent findings of other groups.

(It is interesting to note that recent studies in the NIAID-laboratory of Dr. Michael Kaliner, chief of the Allergic Diseases Section, have linked this pathway of AA metabolism to mucus production in the lungs, another serious problem of asthma.)

In 1979 the chemical identity of SRS was at last established, based on corroborating data from several research groups. The final elucidation was made by Dr. Bengt Samuelsson of the Karolinska Institutet in Stockholm. He also bestowed the name leukotrienes, because the compound develops from leukocytes and h three (tri) double chemical bonds (ene).

Once their structures were known, leukotrienes were purified and synthesized, providing even larger quantities for research. Now their potent role in airway constriction has been confirmed in human studies.

For the future, investigators are confident that what can be synthesized can be blocked. Several pharmaceutical companies already are working to develop analogs of leukotrienes to interrupt the pathway of metabolism and relieve asthma sufferers of their wheezing and shortness of breath. ■

The "Real Secret" of NIMH May Lead Scientists to a Deeper Understanding of the Brain

Jules Asher

In the book the inspired the cartoon feature film "The Secret of NIMH" (currently at local theaters), a fictitious "Dr. Schultz" injects rats with a drug that makes them smart—so smart, in fact, that they figure out how to open their cages and escape to establish their own advanced civilization.

In real life, an NIMH intramural researcher, Dr. Thomas O'Donohue, has been studying alpha MSH, a naturally-occurring brain substance, which when injected in rats enhances their learning and memory—and does the same for humans as well!

O'Donohue's rats given shots of alpha MSH (melanocite stimulating hormone) have hardly waxed brilliant enough to read the instructions on their cages, as their fanciful counterparts do. But in the fantastic event that they ever did escape, they'd likely have their first adventure with lizards in O'Donohue's lab.

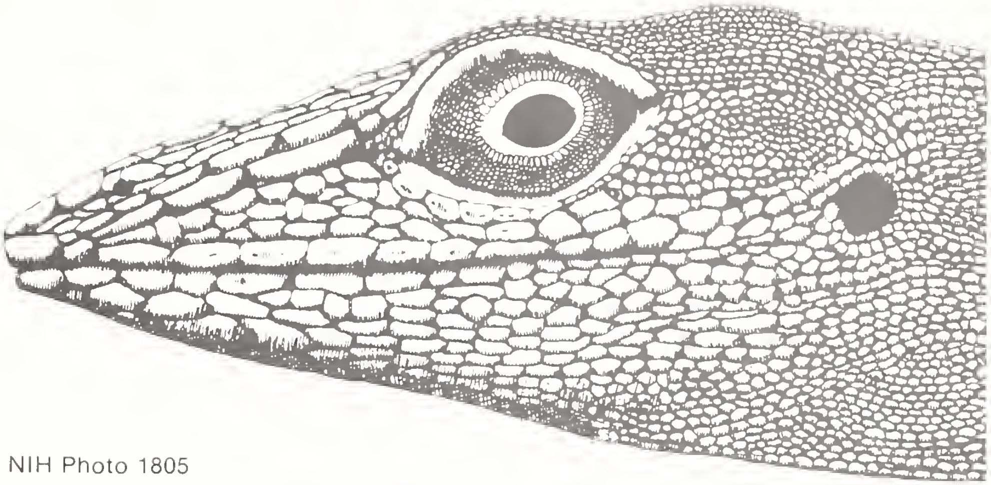
For the real "secret of NIMH" is that the cell on the skin of the chameleon lizard that turns from green to brown holds the key to understanding the cell in the brain that secretes not only alpha MSH but also the brain's own opiate, beta endorphin.

The same chemical mechanism that adapts the chameleon to its environment via camoflage adapts us mammals to ours via a chameleon-like tranformation of the limbic brain cell, the opiomelanotropinergic neuron, from one which induces heightened attention by secreting alpha MSH to one which numbs pain by secreting beta endorphin.

"The chameleon skin cell (melanocyte) serves as a model for the neuron because it is embryonically derived from the same source and contains alpha MSH and beta endorphin receptors," explained O'Donohue, who carried out his experiments in NIMH's Laboratory of Clinical Science with his wife, Dr. Gail Handelmann, and Dr. David Jacobowitz.

"In the brain you have billions of neurons. It's very hard to look at a specific one. An isolated cell like the chameleon's melanocyte allows you to study the molecular mechanisms without all the interfering interactions of other neuronal systems."

Research on the melanocytes has demonstrated the first clear-cut example of a transmitter being fine-tuned by a modulator. In this and related studies with rats, the researchers have also identified enzymes that unlock the secret of the neuron's ability to transform itself. The work is pointing the way to possible development of a new kind of addiction-free painkilling drug.



NIH Photo 1805

Neuroscientists had to drastically revise their theories about how neurons work just within the past decade. They used to think each brain cell secreted just one chemical messenger, or neurotransmitter, which carried the nerve impulse across the synapse to an adjoining neuron.

The work on the opiomelantropinergic neuron adds to mounting evidence that neurons actually release more than one such neurosecretion.

One substance, the neurotransmitter, serves as the primary carrier of the message, while the others may serve as neuromodulators, potentiating or inhibiting the post-synaptic action of the neurotransmitter by binding to related receptor sites on the receiving neuron.

O'Donohue and colleagues showed that in the melanocyte (and perhaps in the neuron) beta endorphin serves as the neuromodulator, while alpha MSH acts as the neurotransmitter. Alpha MSH stimulates the darkening of the chameleon's skin, subject to some fine-tuning by beta endorphin.

In mammals, alpha MSH has been shown to produce additional effects that would surely interest "Dr. Shultz." When injected in rats, it stimulates states of heightened arousal and improves performance on a number of learning and memory tasks. It has been shown to similarly improve performance on tests of visual attention in humans. At high doses, it causes hyperarousal or anxiety.

Beta endorphin plays a major role in the control of pain and at higher doses can cause catatonia, a depressed state which is conspicuously opposite that observed with high doses of alpha MSH.

In his studies with rats, O'Donohue uncovered the mechanism by which the opiomelanotropinergic cell can serve at one moment as an alpha MSH-secreting neuron that stimulates arousal and learning, and at another moment as a beta endorphin neuron capable of inhibiting pain.

The pivotal actor in this elusive process turns out to be an enzyme (opiomelanotropin acetyltransferase) which at once activates alpha MSH and inactivates beta endorphin. This is accomplished via a chemical process called acetylation.

"When the enzyme is turned off, the neuron will secrete active beta endorphin and inactive alpha MSH, so its role will be in producing analgesia," explained O'Donohue. "If the acetylating enzyme is on, the neuron's role is to induce arousal and perhaps anxiety."

He is now trying to develop a drug that will block the enzyme, thereby enhancing analgesia and calming anxiety via presynaptic intervention.

"The problem with existing painkillers like morphine is that they act post-synaptically, chronically stimulating receptors to the point where they demand constant stimulation, resulting in physical dependence and tolerance.

"If a drug can prevent presynaptic inactivation of endorphin by acetylation, the neuron will release all biologically active analgesic beta endorphin and inactive arousal-causing alpha MSH only in times of need. This may avoid chronic receptor stimulation and the ensuing development of tolerance and addiction," O'Donohue concluded. ■

NCI Published Atlas of Mortality for Selected Diseases

Linda Anderson

The National Cancer Institute (NCI) has published a new *Atlas of Mortality from Selected Diseases* mapping the geographic variations in death rates across the United States for 59 conditions. Although the report does not deal with types of cancer, the *Atlas* includes both diseases associated with cancer and other conditions of public health interest.

Scientists of NCI's Environmental Epidemiology Branch have found mapping techniques useful in the study of cancer causation. They have applied these techniques in the new *Atlas* for the use of the general health community.

The authors are Thomas J. Mason, Ph.D., Joseph F. Fraumeni, Jr., M.D., Robert Hoover, M.D., and William J. Blot, Ph.D.

Three general types of diseases are included in the *Atlas*. Some of the diseases have been proven or are suspected to be related to development of cancer, or appear to be different expressions of a common risk factor. Other diseases were selected because they affect a significant segment of the population, and some have been associated with environmental exposures especially prevalent in certain geographic areas.

The conditions include respiratory, digestive, metabolic, neurological and congenital disorders, infectious diseases, and different types of heart disease and related disorders.

Each disease is mapped by the 506 state economic areas (SEAs). The SEA is a unit defined by the U.S. Bureau of Census as a county or group of counties similar in economic and social characteristics.

The maps are based on average annual death rates (deaths per 100,000 population) calculated from deaths in the United States during 1965-1971. The data, obtained from HHS' National Center for Health Statistics, are based on death certificates. Rates were computed separately for white and nonwhites and for males and females. The rates were adjusted to reflect the age distribution of the United States population in 1960.

Summary tables for each disease list the percentile rankings for the mortality rates and number of deaths. Other tables present rates according to five-year age groups. The tables enable the reader to determine the general range for death rates reflected in the color-coded maps. A separate table lists the average annual death rates for each disease and total number of deaths.

The geographic patterns for many of the conditions are consistent with findings of other investigators, and references to some of these studies are provided. The NCI scientists believe the special feature of the *Atlas* is the visual picture it provides of the geographic distribution of death rates for a large number of diseases over the same period.

The maps do not permit inferences about causes of these diseases. Their value, the scientists suggest, may be in identifying geographic areas to study these diseases.

Among the diseases that have been associated with cancer, a consistent pattern of above-average death rates for diabetes mellitus was seen for white males and females from the Midwest through the mid-Atlantic, with clustering particularly in Michigan, Ohio, and Pennsylvania. Above-average rates also were found in southern sections of Louisiana and Texas. In contrast, no consistent pattern was seen for nonwhites. Diabetes has been associated with increased risk for pancreatic and endometrial cancers, and geographic correlations between diabetes and the two cancers have been noted in some studies.

Above-average death rates for gallstones, which are believed to be related to gallbladder cancer, were seen in the upper Midwest (Wisconsin, Michigan, and Illinois), mid-South (Kentucky and Tennessee), and parts of the Southwest. Among nonwhites, the rates were above-average in the Southwest, which is consistent with the recognized susceptibility of American Indians to gallstones and other gallbladder diseases. Mexican Americans also are prone to gallbladder disease, which may account for the high rates seen among whites in the Southwest. Scientists previously have observed remarkable geographic and demographic similarities in rates for gallstone and gallbladder cancer.

Deaths from hepatitis are comparatively rare in the United States, and little clustering of above-average rates was seen except among whites in parts of Texas where death rates for liver cancer are known to be above-average. This relationship may be consistent with that seen between hepatitis-B virus infection and liver cancer in other areas of the world where hepatitis deaths are more common.

For Down's syndrome (mongolism), which is believed to increase risk for leukemia, no geographic variation of rates was seen among whites. Among nonwhites above average rates were seen in parts of the South. In general, however, no pattern was evident, consistent with findings from some recent surveys.

Among diseases which may represent a separate manifestation of the same risk factor for cancer, the scientists found that deaths from diverticulosis and diverticulitis of the intestine occurred at above-average rates in areas of the Northeast, and thus resembled the pattern observed for colorectal cancer. Both diseases have been associated with fiber-deficient diets. In contrast, no geographic pattern of rates was seen for ulcerative colitis, a chronic inflammatory disease of the colon that is known to be a greater risk for colorectal cancer.

For diseases associated with environmental exposures prevailing in certain geographic areas, the scientists found patterns expected from previous experience. The geographic distribution of rates for the pneumoconioses—occupational lung diseases such as silicosis, asbestosis, and black lung—coincided with the location of mining and smelting operations associated with exposures to silica and other dust particles. Clustering of above-average rates was seen in Upper Appalachia extending into Pennsylvania, and in the Rocky Mountain states.

The geographic distribution of rates for rheumatoid arthritis appeared to reflect migratory patterns of individuals with the condition. Above-average rates for the condition tended to be clustered in the southeast, particularly among males, and in the Southwest. In an earlier survey, higher rates for the condition were noted throughout the Mountain states, which suggests the southwestern pattern seen in the *Atlas* may reflect a more recent migration.

The pattern for emphysema death rates also appeared to result at least partly from migratory patterns. A pronounced clustering of above-average rates was seen among whites in the Mountain and Pacific states.

Striking East-West differences were seen in the geographic distribution of death rates for ischemic heart disease (commonly called coronary heart disease) for white males and females. Heart disease is the leading cause of death in the United States, and more deaths are attributed to ischemic heart disease than any other. Above-average rates for whites were concentrated in areas east of the Mississippi River, particularly the mid-Atlantic states, Appalachia, and the southeastern coastal plains. Above-average rates for whites were concentrated in areas east of the Mississippi River, particularly the mid-Atlantic states, Appalachia, and the southeastern coastal plains. Above-average rates were seen for nonwhites in the mid-Atlantic and southern states, but not in areas of the deep South. The pattern for nonwhites in the mid-Atlantic and southern states is considered representative for blacks, who largely comprise the nonwhite population of these geographic areas. The geographic variations resemble those for diseases associated with high blood pressure, which also are included in the *Atlas*. The patterns for cardiovascular diseases are consistent with those described by other investigators, including epidemiologists in the National Heart, Lung, and Blood Institute.

Death rates for kidney stones are low in the United States, but clustering of above-average rates was seen among whites in the southeastern states, particularly along the coastal areas. The reasons are unclear.

Other materials on the *Atlas* include a set of question and answers that provide additional information on the use and interpretation of the publication. A list of National Institutes of Health contracts for the various diseases is provided, as well as definitions of the diseases.

The *Atlas* may be purchased from the Superintendent of Documents, U.S. Government Printing Office, Washington, [illegible]C. 20402. The order number is 017-042-[illegible]57-9. The cost is $18.00 for the United States, Canada, and Mexico, and $22.50 for all other foreign countries. ■

Animal Studies Link Phenobarbitol Usage During Pregnancy to Effects on Reproductive Function in Offspring

James Hadley

Phenobarbital is capable of producing striking and permanent effects on the reproductive function in animal offspring exposed to the drug within the womb, according to research headed by Dr. Sumner Yaffe, director of the Center for Reearch for Mothers and Children (CRMC) at the National Institute of Child Health and Human Development (NICHD).

Although the clinical significance of this finding is unknown, Dr. Yaffe said one thing is clear: phenobarbital should be prescribed cautiously to pregnant women. Further, he added, this finding suggests that other tranquilizers and sedatives taken by women during pregnancy also may have delayed effects on the biologic functions of their offspring. Such drugs currently represent the major group of drugs prescribed to pregnant women.

Dr. Yaffe discussed the results of his research during an NICHD Pediatric Grand Rounds seminar entitled "Effects of Intrauterine Phenobarbital Upon the Reproductive Function," held recently at the National Institutes of Health.

Before joining the Institute last fall, he headed a team of University of Pennsylvania researchers, supported by NICHD. The team conducted three studies which demonstrated a variety of permanent reproductive dysfunctions in the male and female offspring of rats injected with phenobarbital, a hypnotic sedative commonly used during pregnancy. In all the studies, the litter size and birthweights of the test offspring were similar to those in the control group.

The studies showed, however, that a dosage of phenobarbital given to pregnant rats during an eight-day period (12 to 19 days of gestation) produced in their offspring significant effects such as suppressed body weight, infertility, and delays in the onset of puberty.

In male offspring, the drug causes a delay in testicular descent, infertility and decreased production of sex hormones, testosterone and gonadotrophin. However, the age of onset of puberty did not change.

To investigate the reason for the reproductive dysfunction, the researchers analyzed hormone concentrations in plasma and found significant increases in both estrogen and progesterone and a decrease in the concentration of luteinizing hormone which is released by the pituitary gland. No differences were observed in the brain, hypothalamus and pituitary.

These findings, said Dr. Yaffe, clearly show that prenatal exposure to phenobarbital produced long-term defects in the onset of puberty, menstrual cycle and fertility—all of which may be related to altered concentrations of both sex and gonadotropic hormones which work hand in hand to signal to the brain when to release LHRH (luteinizing hormone-releasing hormone). LHRH controls the pituitary gland which in turn regulates the testes in males and ovaries in females to produce testosterone and progesterone, respectively.

The studies also determined that rat exposure to phenobarbital only during the period of fetal neuroendocrine development (17 to 20 days of gestation) can produce the same adverse effects. The results also suggest that the drug need not be present during earlier periods of sexual differentiation to produce its effects, indicating that phenobarbital does not interfere with gonadal or ductal differentiation.

The fact that postnatal administration of phenobarbital can affect reproductive function, even though the development of sexual organs has been completed, lends further support to the theory that the drug's action is dependent upon neuroendocrine differentiation, said Dr. Yaffee. More research is needed to fully understand why this happens. Only after further animal research and human studies can the clinical relevance for humans be determined, he cautioned.

Results were published in *Science* (Vol. 208, pp. 508-10), *Pediatric Pharmacology* (Vol. 1, pp. 55-62) and *Pediatric Research* (Vol. 15, pp. 1488-1491). Co-investigators were doctors Chhanda Gupta, Bernard H. Shapiro and Robert Sonawane. ■

Sickle Cell Disease—Research Findings Point to New Solutions

Laura Winston

Sickle cell anemia touches the lives of more than 2 million people in the United States. These people may have relatives or suffer themselves, from the painful and damaging effects of the disease.

The effects are diverse, in fact the disease takes so many forms from mild to severe, that sickle cell anemia has been called the "great imitator" of other diseases.

Sickle cell anemia occurs because of a mistake in the genetic code. A person with sickle cell disease has an abnormal hemoglobin (sickle hemoglobin) as a result of this error.

Hemoglobin, which is the oxygen carrying component of the red blood cell, is made up of four protein chains—two alpha and two beta chains. Each protein chain is made of amino acids linked together "like beads on a string."

In most people the sixth amino acid in the beta chain of hemoglobin is called glutamate. However, in a person with sickle cell anemia, valine replaces glutamate.

This change in the sixth amino acid of each beta chain is responsible for such diverse symptoms as pain crises, arthritis, stroke, and heart failure.

Sickle cells look normal in the oxygenated blood flowing through the arteries. In the capillaries, the red blood cells give oxygen to the tissues (becoming deoxygenated) and receive carbon dioxide which is carried to the lungs to be exhaled during respiration.

It is in the deoxygenated blood that the sickle hemoglobin in the red blood cells becomes aggregated. The blood cell becomes increasingly rigid and takes on a sickle shape.

Many sickle cell patients experience sickle cell crisis periodically. Crisis occurs when the rigid cells get stuck in small blood vessels such as the capillaries, blocking the flow of blood.

Eventually, larger blood vessels become obstructed. The result is extreme pain in the chest, back, abdomen, joints and sometimes other parts of the body.

Pain crisis may last a few hours or up to three weeks. Some patients have a crisis several times a year and some never have a crisis.

Why crisis occurs is not really known. Often stress, infection, dehydration or altitude exposure cause sickle cell crisis but sometimes crisis seem to occur for no apparent reason at all.

Another problem for people with sickle cell disease is called hemolytic anemia. Anemia occurs because the sickle cells break down faster than normal red blood cells.

People with hemylotic anemia may feel dizzy, weak and tired. If they have severe anemia they may require blood transfusions.

The third and very serious effect of sickle cell anemia is organ damage from long-term vessel obstruction. Any organ in the body can be affected.

Consequently, the disease may cause stroke, lung damage, heart or kidney failure, leg ulcers, arthritis as well as other disorders.

Another problem is that children develop infections easily. This occurs because often the body's immune system is affected. Infection may lead to sickle cell crisis.

Although the sickle cell anemia story sounds grim, there is hope. Scientists are beginning to understand the underlying cause of the disease. Modern treatment of the complications of the disease is usually successful and many people live today who would have died a few decades ago.

"We don't have good data on lifespan in sickle cell disease," said Dr. Clarice Reid, Chief of the Sickle Cell Branch of the National Institutes of Health. She said that the life span of people with sickle cell disease may be shortened but "many live long productive lives."

Her "subjective perception" is that there has been "a change in the quality of life" for these people. Life is improving for sickle cell patients because much research is being done.

Since hemoglobin aggregation, cell sickling and rigidity are believed to occur only in the capillaries and veins where the blood is deoxygenated, new research by Dr. Alan Schechter may be important.

Schechter and Dr. Constance Noguchi, at the National Institutes of Health, found that sickle cell anemia may be a misnomer. The sickle shape of the red blood cell may not be an important factor in the disease.

Schechter was working with antisickling agents when he realized he didn't understand "enough about what was happening to hemoglobin in the red cell."

He began to study the biochemistry of hemoglobin. Using a new technique involving nuclear magnetic resonance spectroscopy, developed in part by Dr. Dennis Torchia, an investigator at the National Institutes of Health, he was able to actually measure aggregation of hemoglobin in the abnormal red blood cells.

He found that hemoglobin aggregated not only in deoxgenated blood, but also in blood high in oxygen. In fact, some aggregation occured in blood almost 100 percent saturated with oxygen.

Schecter said that "sickling is largely a laboratory phenomenan and sickling, *per se* may not be that important in patients." What is important is that sickle cell anemia is a continuum. The flexibility of the cell changes continosly, not just in the deoxygenated blood of the capillaries and veins.

Schechter said he now believes that the abnormal red blood cells begin to aggregate in the arterial part of the circulation, and not just when the sickled cells become fully deoxygenated.

"We will have to rethink a lot of therapeutic approaches" if Schechter's findings are correct, Reid said. Therapy will have to work on the arterial side of the circulation.

New treatment could include drugs vasodilators—that make the arter wider so the blood cells can flow smoothly through them. A drug that decreases the amount of hemoglobin aggregation in the red blood cells should be effective too, Schechter said.

He said that new research is necessary to develop possible treatment. Although Schechter's research is promising, there are other National Institutes of Health researchers looking at other aspects of sickle cell anemia.

Here are a few examples:

- Researchers in genetic engineering are studying how the single mutation in the beta chain of hemoglobin may someday be altered.
- Reid said that sickle cells stick to the insides of the blood vessel more than normal cells. Some researchers are studying how greater adhesion leads to more severe cases of sickle cell anemia.
- Other researchers are studying hemoglobin produced by the fetus. This fetal hemoglobin carries oxygen as effectively as normal hemoglobin, even in people with sickle cell anemia. If there was a way for people with sickle cell anemia to keep producing fetal hemoglob they whould have less chance pain crisis, anemia or organ damage.

Sickle cell anemia is an unusual disease. It does not exist in laboratory animals, which makes it difficult to study.

In 1972, ten sickle cell centers were set up throughout the United States, with funds from the NIH. They are special because in one place, scientists conduct basic and clinical research and the public receives screening, counseling and education.

"Research findings can be readily transferred to application at the centers," said Reid. Scientists, the public and health professionals are very enthusiastic about the centers.

"They are seen as a prototype in ways to conduct research and provide health care in special disease areas," Reid said.

The services at the centers are free to the public. Medical and other college students conduct projects at the centers throughout the year and there are summer science programs.

The sickle cell centers have contributed to the public and professional understanding of the disease through education. In addition, many health programs in the community are sponsored by the centers.

The result has been a tremendous improvement in patient care because health professionals know more about the disease.

Most people with sickle cell anemia have access to the health care system because of the centers. They are diagnosed and treated earlier. They take better care of their health because they understand the need for prevention of infection and stress.

The centers provide counseling as well as education and screening. "Any chronic illness has an impact on a person's life," Reid said.

The person with sickle cell anemia has to deal with the perceptions of the people around him. He may have low self esteem. Counseling can help the person learn to cope with all of these problems.

A serious disease is hard for anyone to cope with, especially a child. The coordinated approach to sickle cell research and treatment at the centers as well as research throughout the country, is helping.

Someday, painful sickle cell crisis, seen in so many hospital emergency rooms, may be a thing of the past. ■

NIMH Scientists Find Drug Enhances Effects of Acupuncture

Jules Asher

Two Chinese neuroscientists working at NIMH have prolonged the analgesic effects of acupuncture by chemically blocking the metabolic breakdown of a brain opiate.

The study with rats, conducted by Drs. Tian Tang and Anzhong Zhang of the Peoples Republic of China, is the first to demonstrate a drug enhancement of acupuncture effects, according to Dr. Erminio Costa, chief of the Laboratory of Preclinical Pharmacology, where the visiting fellows have been working.

It adds to mounting evidence that the brain's own opiates—the endorphins—are released by acupuncture and play an important role in the effects of this Chinese treatment.

Tang of the Bejing Medical College and Zhang of the First Medical College of Shanghai came to the U.S. to follow up on earlier Chinese findings that acupuncture triggered release of an endorphin-like substance in the rat brain.

They also built upon earlier work by Dr. Hsiu-Ying Yang of the NIMH lab. She discovered that the drug captopril (used to treat high blood pressure) can inhibit the enzyme in brain which breaks down the endorphin-like heptapeptide (Met^5 enkephalin-Arg^6 Phe^7).

Captopril was originally synthesized specifically for inhibiting the enzyme that breaks down angiotensin, a substance in blood that constricts veins and arteries. This enzyme is chemically similar to the enzyme in brain (dipeptidyl carboxypeptidase) which Yang found breaks down the heptapeptide and can also be inhibited by captopril.

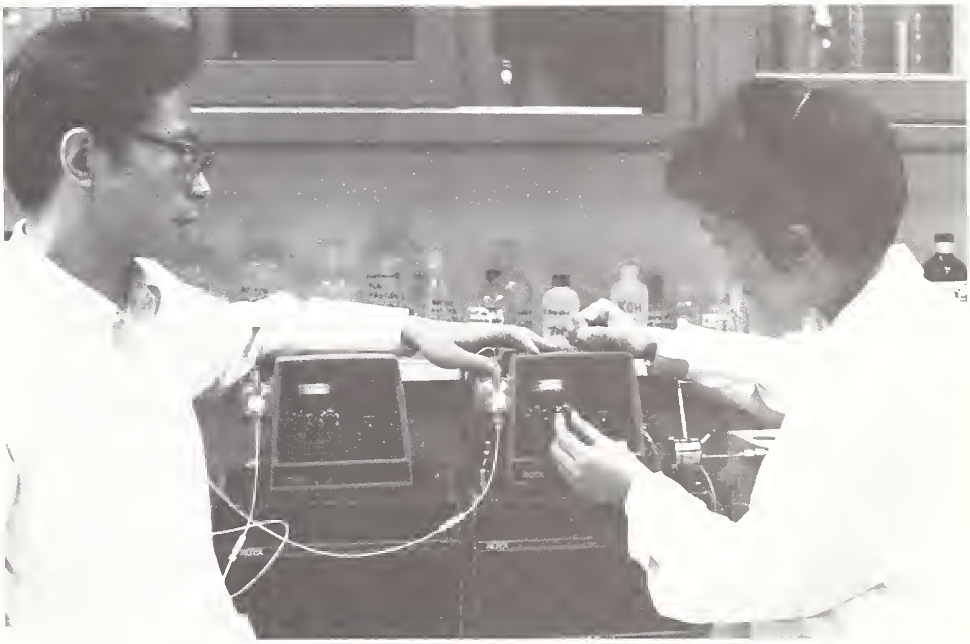
The two mainland scientists hypothesized that captopril might, by blocking its metabolism, "protect" the naturally occuring opiate, allowing it to pool in the brain and thereby aid acupuncture.

They injected the drug directly into rat brains and then administered electroacupuncture. A mild electric current was passed—via needles—through the animals' hind legs for 10 minutes. The results were dramatic.

Pain thresholds increased significantly, and the analgesia was prolonged markedly—lasting for 15 minutes after the acupuncture treatment ended. In control animals that received acupuncture alone, the effect wore off much sooner.

A marked accumulation of the endorphin-line heptapeptide was found in the brains of the experimental animals. Significantly, injection of the opiate antagonist naloxone immediately reversed the effects of the drug-enhanced electroacupuncture, suggesting a pivotal role for the endorphin system.

Work is now underway to modify captopril so that it might pass through the blood-brain barrier, said Costa. This may eventually permit use of the drug as an adjunct to clinical acupuncture treatment.



NIMH scientists use high pressure liquid chromatography for separating and identifying opiate peptides involved in acupuncture.

NIH Photo 1802

Unusual Hyperactivity Study at NIH is Fun and Beneficial to Patients

Laura Winston

An unusual hyperactivity study at the National Institutes of Health is fun for the subjects and beneficial to the researchers. Dr. Alan Zametkin, associate, Biological Psychiatry Branch, National Institute of Mental Health studies the causes of hyperactivity in children by comparing the effects of standard medications to new drugs.

There are four children in the program at a time. All are boys, because the disorder is four times more common in males. None have serious neurological problems.

"The kids love it here, we have to scrape them off the walls to get them out," Zametkin said. The children come to the NIH clinical center, Monday through Friday, for three to six weeks. A special van picks them up in the morning and takes them home at night.

They attend school in the morning and participate in structured activities during the rest of the day, including library activities, occupational and recreational therapies. The clinical center features a gym, sun deck and playroom for the boys.

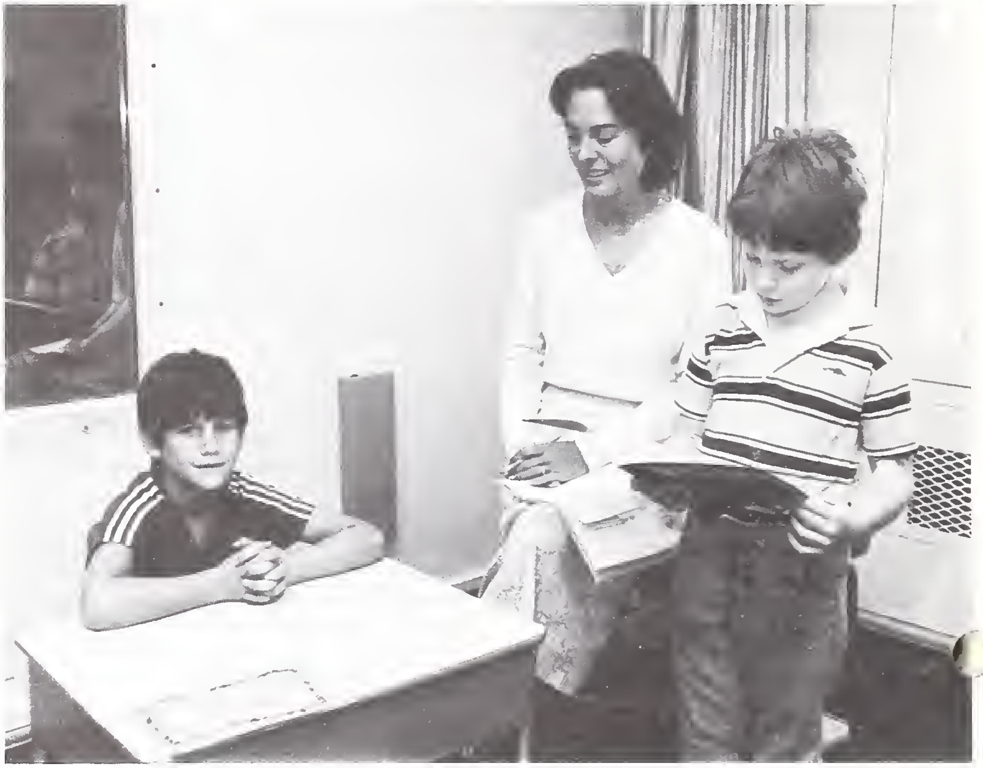
The children get a free "neurological, psychiatric, educational and medical evaluation" to make sure there aren't any obvious medical or psychological reasons for their hyperactivity, Zametkin said.

He said that stress at home or abusive parents may cause some children to act like they are hyperactive.

Once they are admitted to the program, the boys are given either a hyperactivity drug or a placebo (a substance that has no real effect). Some of the drugs are standard medications such as dexadrine, a stimulant. These drugs help hyperactive patients, but medical researchers do not know why they work.

The standard drugs are compared with new medications. Researchers "have a better idea about what these new medicines do to the brain," but do not know if they will be helpful in cases of hyperactivity, Zametkin said.

While they are on the drugs, the children are observed in the classroom and in play activities. They take speech, language, memory and learning tests and their parents and teachers rate their behavior. The boys wear activity monitors on what resembles toy army belts. The monitors are "solid-state devices that record how active the kids are every 15 minutes," Zametkin said.



Although fun activities are an important part of the hyperactivity program, the children also attend school in the morning. The classes are small, emphasizing individual attention and care.
NIH Photo 1803

While the children are on medication, Zametkin takes urine and blood samples and compares them with the changes in the children's behavior. He said that some of the changes observed in the children are due to the strict behavior modification program they must follow while in the study.

The children get play money for every half hour they behave appropriately and penalties for inappropriate behavior. They can spend the money on free time, time in the playroom and prizes.

Improvement is also due to the individualized attention these children get while in the program. Attention they may not be getting at home. "These kids get a lot of individual attention, which also produces improvement," Zametkin said.

A strict behavior modification program may also be very helpful. However, "it takes a tremendous amount of effort by parents and teachers" to make such a program work. Often parents don't have the motivation, Zametkin said. In addition, kids who are often punished for hyperactive behavior by parents and teachers may have low self esteem.

The nurses and doctors make the children feel really wanted here. The children feel they are "not bad all the time, they don't feel constantly put down," Zametkin said.

At the end of their participation in the program, the children are given the appropriate medicine if they need it. They may have referrals to counselors if they have special problems.

Some children may stay on behavior modification. Their success depends on how well their parents follow through with the staff's recommendations. Some children do very well and others go back to the same behaviors they had before the study, Zametkin said.

"Our basic purpose is to do research, but in the context of research we give good treatment, evaluations and recommendations," he added.

For additional information contact D Alan Zametkin at (301) 496-1891 or fo referrals contact Dr. Walt Sceery at (301) 496-6081. ■

Insulin Self-Suppression is Less Effective in Obese People

Laura Winston

High levels of insulin in the blood inhibit the body's insulin-secreting mechanism according to researchers at the National Institute on Aging. This process is less effective in obese individuals.

"We have conclusively, elegantly, and convincingly shown that insulin indeed can suppress its own secretion," said Dr. Dariush Elahi, of the Clinical Physiology Branch, Gerontology Research Center, National Institute on Aging in Baltimore.

The beta-cells of the pancreas produce the hormone insulin, which the body uses to regulate glucose (sugar) levels in the blood.

Insulin stimulates the body to move glucose from the blood into muscles cells and adipose tissue. Without this hormone, the body could not use glucose properly.

Elahi and his associates conducted studies on male and female caucasian and Pima Indian volunteers. To test for differences between obese and lean people, they divided their subjects into three groups: lean caucasian, obese caucasian and obese Pima Indian.

Pima Indians are noted for having very large numbers of obese people. Furthermore, they have "the highest recorded prevalence of diabetes."

In the study, each person fasted for 12 hours before the study began. Elahi then inserted a polyethylene catheter into a vein and gave the subjects 90 minutes of continuous insulin until they had very high insulin blood levels.

The same vein was used for glucose infusion to keep each person's blood sugar at normal levels.

Elahi measured the amount of insulin produced by the body by measuring levels of C-peptide (a molecule that is split off when insulin is formed). Every time the pancreas produces insulin, it produces C-peptide.

"C-peptide can only be produced by the body," Elahi said. By using C-peptide, he was able to distinguish between the insulin he infused and the insulin the pancreas secreted.

Elahi found that that high blood levels of insulin did inhibit further production of insulin by the pancreas. In obese people, however, insulin's inhibitory actions were not as effective.

"In the obese state, insulin does not self-regulate its own secretion as well," Elahi said. The obese people, especially the Pima Indians, normally had higher levels of insulin in the blood than the lean subjects. When the infusion began, all three groups blood levels of insulin rose.

In response, the beta-cell production of insulin was suppressed in all subjects, but the obese people still produced amounts of insulin that were higher than normal. Lean people were also able to suppress insulin secretion longer.

Elahi explains this finding by suggesting that obese people may have an abnormally large amount of beta-cells in the pancreas. Another possibility is that there are a normal amount of beta-cells, but they may be secreting too much insulin.

Another finding was that lean subjects initially had lower levels of blood sugar because they used their insulin efficiently. They required more infusions of glucose during the study to keep their blood sugar levels normal.

Obese subjects had higher levels of blood sugar, reflecting the well-known finding that fat people have a decreased sensitivity to insulin.

Dr. Elahi suggests that further studies should be done in this area, particularly with obese subject's inability to suppress insulin secretion efficiently.

This study was supported by a grant from the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases. ■

New Drug Therapy May Provide Hope for Infants with Blood Vessel Defect

Laura Winston

A drug called Indomethacin offers a simple and usually effective means of treating patent ductus arteriosus, a blood vessel defect found in premature babies. Researchers at the University of California, San Francisco found that Indomethacin therapy may prevent the problem of patent ductus arteriosus, if the drug is given as soon as the problem is diagnosed. Their work was supported by a program project grant from the National Heart, Lung and Blood Institute.

Dr. Mahony and her associates studied 47 premature infants who showed the beginning signs of patent ductus arteriosus (PDA).

Normally in the fetus, there is a tube (the ductus arteriosus) that connects the aorta—which delivers blood from the heart to the body, to the pulmonary artery—which delivers blood from the lungs to the heart.

At of shortly after birth fetal circulation normally changes to the adult pattern with closure of the ductus. In premature infants, closure may be delayed or may not occur and this can place serious burdens on the infant's circulating system.

Infants with this problem have trouble breathing. They may have other problems such as hemorrhages in the brain and sometimes they die. The problem is usually corrected by surgery. However, Mahony's research indicates that surgery may not always be necessary.

Mahony found that Indomethacin helped "low weight" premature infants effectively, if they were given the drug as soon as PDA was diagnosed. Most of these babies did not need surgery and had no complications from PDA.

Previously, infants were treated with Indomethacin, but only after problems had developed.

The researchers selected the premature infants from the intensive care units at the University of California, San Francisco medical center and Mt. Zion Hospital and medical center. All the infants had evidence of PDA without any problems from the disease.

The doctors detected heart murmurs (abnormal heart sounds) and used other tests to make a diagnosis. They divided the babies into two groups. One group weighed less than 1000 grams (2.2 pounds) and the other group weighed more than 1000 grams.

Half of the infants received the drug Indomethacin. The doctors and nurses caring for the babies didn't know which infants had received the drug.

Mahony found that Indomethacin helped the tube (ductus) to close in the infants who weighed less than 1000 grams. Few of these infants needed surgery and they had fewer complications, gained weight and needed less oxygen therapy.

In contrast, 83 percent of the infants who did not receive the drug developed problems, such as blood flowing back to the lungs and difficult breathing.

Indomethacin did not help the infants who weighed more than 1000 grams because many of these infants had spontaneous closure of their ductus and did not develop problems. More research in this area is needed, according to the researchers.

Surgery is especially risky for "low weight" infants. Early Indomethacin treatment may decrease or eliminate the need for surgery in patent ductus arteriosus.

This research was reported in the March 4, 1982 issue of the New England Journal of Medicine. ■

New Computer System Interprets Results from Heart Research

Charlotte Armstrong

A recently installed ECG management system has begun to record, interpret, and store electrocardiographic tracings made in the Clinical Center. Eventually the system will process all ECG's made in the Clinical Center and NIH's Occupational Medical Service.

Clinical Center planners selected a versatile computer system with the intent of modifying it to meet the special research needs of the Clinical Center. Interest in computerized ECG analysis began in the early 1970's with collaborative studies by the National Heart, Lung and Blood Institute (NGLBI) and the Division of Computer Research and Technology (DCRT).

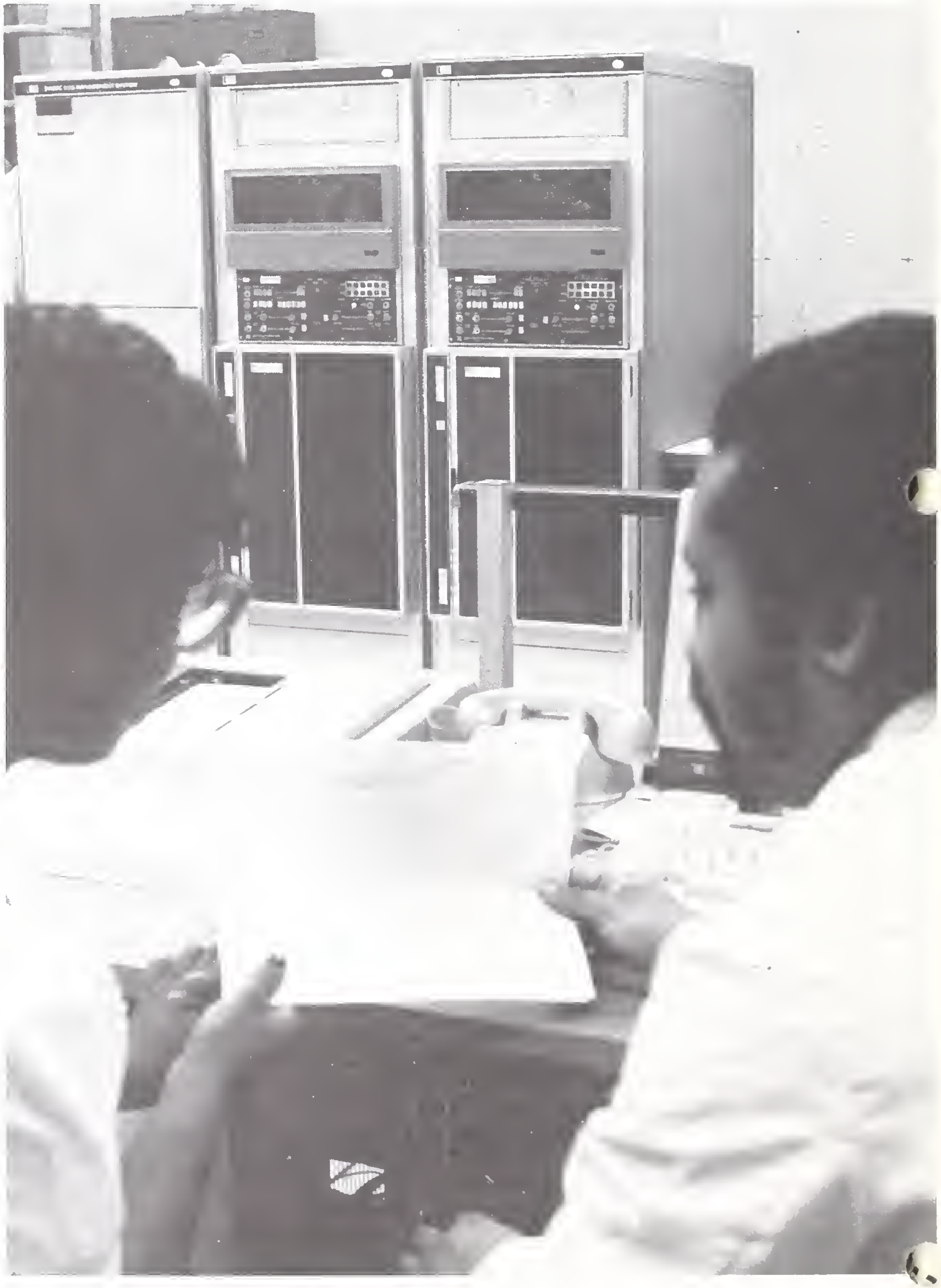
Dr. Sebastian Palmeri, Chief of the Cardiology Consultative Service, and Director of the ECG Laboratory, NHLBI, and Kenneth Kempner, DCRT, project officer for the computerized ECG system, now oversee its daily operation.

The essential advantage of computerized ECG processing is the conversion and recording of an ECG tracing in a computer-readable format. This provides the basis on which a computer program can be written to interpret electrocardiographic data. It also enables a researcher to use the computer to manipulate ECG data, and to rapidly search large numbers of ECG records for certain kinds of information. The computer can store ECG records which can be easily and rapidly retrieved for serial comparison, teaching purposes, and most important for the Clinical Center research.

An ECG tracing illustrates the passage across heart tissue of the electrical impulse that stimulates heart contraction. This electrical impulse is to some extent transmitted through fluids around the heart and to the body surface. The periodic variations in voltage in the heart can be measured by placing electrodes on the skin and measuring the voltage difference between two electrodes at a time. Conventional electrocardiographs use a system of 10 electrodes. The ECG takes a series of measurements of the difference in voltage between 12 predertermined combinations of the 10 electrodes. Thus, the ECG is actually a set of 12 measurements of the variation in voltage over time at different locations on the body.

When the electrical signal from the electrodes is relayed to the ECG computer, the computer samples the voltage readings 250 times a second. The computer can store these measurements, use them as the basis for its own interpretation of the ECG, and can reconstruct an ECG tracing which is exactly analogous to a tracing made directly from the ECG electrodes. All of these functions can be carried out almost simultaneously.

To interpret the data it compiles from the ECG, the computer conducts a series of measurements of the amplitude, shape, and frequency of the electrical waves in the heart and the relationship of different



Computer operators edit each day's ECG's, incorporating the reviewing physician's conclusions in each computer-generated ECG report. The ECG computer is in the background.

NIH Photo 1804

characteristic waves to each other. Finally it compares these measurements with a set of criteria for various heart conditions and selects one or more diagnoses based on these criteria.

The computer prints the ECG tracing, interval and amplitude measurements and preliminary interpretation. A physician must then "overread" every computer-generated ECG interpretation to verify its accuracy and to make comparisons to the patient's earlier ECG's not recorded by the computer. The tracing with diagnosis is then reprinted with a notation that a physician has reviewed the electrocardiogram. Changes made by the physician are identified with asterisks. A report, in the standard format generated by the Clincal Center's Medical Information System (MIS), is printed by the computer.

As with a conventional ECG, this computer-generated and physician-edited record goes into a patient's medical records. The information is also stored by the computer, which, used as a research tool, is able to search, recall, and manipulate data as research protocols require.

For example, a researcher looking for drug effects on the heart can search the computer's records for all patients treated with a certain drug. This might involve cardiac medications or non-cardiac drugs such as cancer chemotherapeutic agents. In the future, Palmeri and Kempner predict application of the computer in other types of research, such as the more precise estimation of the size of a myocardial infarct or the severity of left ventricular hypertrophy. The system's designers requested features that would provide for future adaptability to such projects. One of these features, says Kempner, is compatibility of the Hewlett-Packard system with other computers in the Clinical Center such as the MIS system.

One of the digital tape recorders in the system will provide an audit trail, by which every form of information or activity that occurs within the system during the day is recorded and stored independently of the computer's other operations. In the case of any breakdown, these records can be searched to trace problems and to reconstruct the day's tracings.

The programs with which the computer now operates can be modified and new programs can be written to accomodate the requirements of future research protocols. Lee Freeman, a DCRT computer programmer, will work with the ECG computer operators, Brenda Butler and Stanley Bristol, to implement and test new research programs.

Computerized ECG processing at the Clinical Center promises to provide physicians responsible for patient care with a useful adjunct to other diagnostic and monitoring techniques and to provide researchers with a new and versatile tool with which to study cardiac function. ■

Abnormal Immune Response in Kaposi's Sarcoma Due to Nitrite Use

Linda Anderson

In a study of 15 healthy homosexual men, scientists have observed abnormalities of the immune system among homosexual men. The scientists suggest that nitrite-induced immune suppression may occur in the face of repeated challenges to the immune system from viral infections, and predispose homosexual men to several diseases.

The findings were reported in *The Lancet*, February 20, 1982, by Drs. James J. Goedert, Dean L. Mann, Mark H. Greene, Joseph F. Fraumeni, Jr., and William A. Blattner of the National Cancer Institute; William C. Wallen of the National Institute of Neurological and Communicative Diseases and Stroke; Douglas M. Strong and Carolyn Y. Neuland of the Naval Medical Research Institute and Uniformed Services University of Health Sciences; and Christine Murray of Biomedical Research Institute, Rockville, Maryland.

Kaposi's sarcoma is a rare skin cancer that usually occurs in the United States among elderly men or, in a severe form, among persons with weakened immune systems (kidney transplant patients, for example). However, an increasing number of cases have been reported in the past few years among homosexual men. Opportunistic infections are also seen among patients with weakened immune systems. Microorganisms that would not be able to multiply in people with normal ability to fight infections can become life-threatening when given the opportunity to infect people with diminished immune defenses.

Some investigators have suggested that nitrite inhalants, which have grown very popular among homosexual men as sexual stimulants, may be immunosuppressive and play a role in the occurrence of the cancer and opportunistic infections such as *Pneumocystis carinii*, an unusual pneumonia.

The investigators collected clinical, virological, and immunological data on 15 homosexual male volunteers in apparent good health. Similar data also were collected on two Kaposi's sarcoma patients. Of the 15 healthy men, 8 were regular amyl or butyl nitrite users (1 to 20 times per month). Seven of the eight men has low helper/suppressor (H/S) T-lymphocyte ratios. Helper and suppressor T cells are types of white blood cells that mediate or modulate immune reactions, and smaller numbers of helper T cells, which aid in antibody production. Of the seven healthy men who did not use nitrites (fewer than 10 times ever), only one man had a low H/S ratio.

Fourteen of the 15 men had antibody to cytomegalovirus (CMV), a type of herpesvirus, indicating past exposure to the virus. Antibody levels were similar for both nitrite users and nonusers. These data thus suggest that CMV infection alone is not sufficient to produce the T cell abnormalities found in this study. Researchers have suspected that CMV may play a role in the occurrence of Kaposi's sarcoma and opportunisitic infections. The investigators suggest the data provide preliminary evidence that nitrite-induced suppression of the immune system, together with repeated infections with CMV or perhaps other agents, predisposes homosexual men to the diseases. The body may become "hyperimmunized" by being repeatedly stimulated to defend itself against viruses, the investigators suggest, and thus susceptible to nitrite-induced immunosuppression.

Both Kaposi's sarcoma patients in the study has used nitrites regularly. They had antibody against CMV and low H/S ratios before treatment with chemotherapy. *The Lancet* article provides the first immunologic data published on Kaposi's sarcoma patients.

Several aspects of the study should be noted: First, the study group was extremely small. Second, other studies have suggested that frequency of nitrite use may be correlated with number of sexual partners. The potential significance of this association for development of Kaposi's sarcoma and opportunistic infections could not be definitively studied here. And, third, this study, like others, focuses on immune dysfunction as a key factor in the development of the cancer. It is possible that some other factor associated with nitrite use, but not the drugs themselves, causes the immunosuppression and leads to the development of cancer and opportunistic infections. ■

Diagnostic Aid Detects Latent Heart Disease Before Symptoms

Jan S. Ehrman

Thallium scanning and electrocardiogram (ECG) monitoring during exercise, a relatively safe, non-invasive technique used to determine which regions of the heart may be deficient in circulation, is providing investigators at the National Institute on Aging's Gerontology Research Center (GRC) with a reliable tool for detecting individuals afflicted wth coronary artery disease (CAD), but who show no noticeable signs or symptoms of the disease.

CAD is characterized by a significant narrowing or blockage in one or more of the three coronary arteries which normally supply the heart muscle with blood. The blockage is caused by cholesterol-laden, calcific deposits in the coronary arteries which ultimately limit blood flow to the heart. Hypertension, smoking, and elevated blood cholesterol levels are the three major risk factors which contribute significantly to the development of CAD.

Identifying persons with latent CAD proves to be a major challenge for physicians, who realize that in some people no outstanding characteristic such as pain exists.

In the past, symptoms and ECG criteria at rest provided researchers and physicians with a low prevalence rate for CAD, compared with autopsy data, and were particularly ineffective in identifing those with the disorder who were asymptomatic. A history of angina, infarction, or resting electrocardiographic abnormalities positively identifies only about half of these individuals.

However, recent studies conducted by physician-scientists at the GRC and The Johns Hopkins University have shown that thallium scans in combination with exercise ECG can provide a more effective and accurate means to identify latent CAD. The Hopkins team administered thallium-201 intravenously during maximal treadmill exercise to 233 randomly selected subjects in the Institute's Baltimore Longitudinal Study of Aging (BLSA) in whom ECG was also monitored. The combination of exercise ECG and thallium scintigraphy (scanning) evinced a much higher prevalence of CAD in each age cohort than did standard "resting criteria" previously mentioned. Among the 70- to 79-year-old group, for instance, resting criteria identified CAD in 22 percent of individuals. However, the addition of individuals with a positive exercise thallium scan, plus an abnormal ECG in conjunction with resting criteria, resulted in a CAD prevalence of 56 percent in this age group.

Exercise ECG involves a regimen of walking on a sloped treadmill while the ECG is continuously recorded. The treadmill is gradually raised, increasing the difficulty of the task. The ECG recording indirectly indicates whether or not the heart can withstand the stress of exercise by maintaining an adequate supply of blood.

Thallium scans are relatively simple to administer and monitor. After intravenous injection during peak exercise, thallium travels to the heart, propelled by the flow of blood. The thallium, a radioactive tracer substance, produces an image of the cardiac muscle, which is then transcribed on a computer screen. Those areas of the heart which do not illuminate with thallium are deficient in circulation, due to some blockage or constriction in the coronary arteries.

According to GRC cardiologist Dr. Jerome Fleg, the exercise ECG yields a diagnostic sensitivity of about 65 percent for detecting CAD in a population with chest pain. When the exercise ECG is used in conjunction with the thallium scan, however, the sensitivity improves significantly, identifying 80 to 85 percent of persons with CAD.

Exercise electrocardiography (pictured), when used in conjunction, with thallium scanning provides investigators with a safe, reliable method for detecting asymptomatic coronary artery disease.

Besides its diagnostic accuracy and the relatively pain-free nature of the exercise, stress thallium scans and ECG have other added advantages. They usually can be administered over a short period of time, requiring no hospitalization or extended treatment and carry minimal safety risk. These advantages contrast with the disadvantages of using angiography, the definitive test for diagnosing coronary heart disease. Angiography involves inserting a plastic tube into the heart, normally requires hospitalization, and carries with it a small, but definite mortality risk.

At the GRC, results obtained from the stress thallium scans and exercise ECG give researchers vital medical information needed to study the effects of disease, as compared to the effects of age, *per se*, on the cardiovascular system.

Clinically, stress thallium scans in conjunction with exercise ECG offer physicians a practical and reliable way to detect asymptomatic CAD. This unique diagnostic aid could have a significant bearing on the health and welfare of heart patients, leading to earlier recognition and treatment and a more optimistic outlook for those afflicted with this disorder, the leading cause of death in the U.S. ■

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Ms. Maureen Napper
National Institutes of Health
Building 31, Rm. 2B-10
Bethesda, Maryland 20205

Ms. Maureen Napper:

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News&Features from NIH

October 1982

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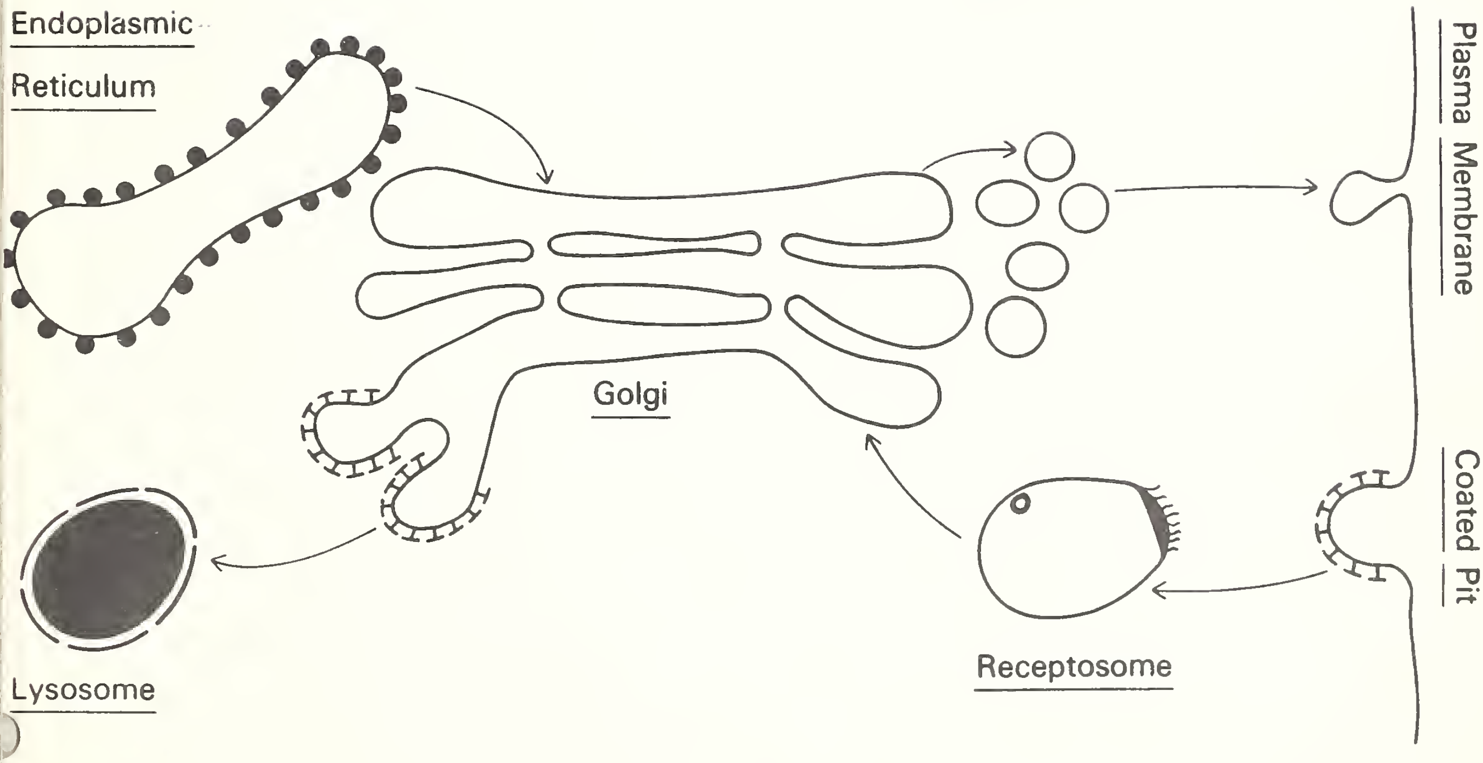

The Golgi apparatus directs the flow of intracellular proteins, processes the ligands, and may be responsible for recycling the receptors back to the surface of the cell.

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

NIH PHOTO 1806

News&Features
from NIH

OCTOBER 1982

An NIEHS research team is currently trying to identify certain drugs and environmental chemicals that may cause birth malformations, especially facial/cranial defects.

The researchers administered various drugs and chemicals to mice to learn the biochemical and morphological sequence of events that leads to malformations in humans. See page 9.

NINCDS scientists are currently trying to restore hand control to spinal cord injury victims by electrically stimulating the paralyzed muscles.

The device is small enough to be implanted in the paralyzed patient's thumb and forefinger muscles and is stimulated by the movement of the patient's shoulder. See page 8.

NIH researchers recently developed their own brain scanner to produce even clearer, more precise pictures of the brain, a necessity when studying neurological and communicative disorders.

This new improved scanner will allow the researchers to "see" the pathological involvement in patients with Alzheimer's disease, epilepsy, and amyotrophic lateral sclerosis. See page 4.

Mayo Clinic dermatologists have been following over 900 patients with pityriasis rosea, a skin disease that is most prevalent among women and people between the ages of 10 and 35.

They found that many of their patients suffered an acute infection within three months of contracting the skin disease and that often a family member also came down with the itching, inflammation and red patches associated with pityriasis rosea. See page 6.

Endurance or aerobic exercise training seems to benefit kidney failure patients, according to a recent study conducted by NIADDK grantees.

Data revealed that a regularly scheduled aerobics program resulted in lowering blood pressure, correcting lipid and fat metabolic problems, and reducing anemia in patients who were using artificial kidneys or otherwise being dialysed. See page 5.

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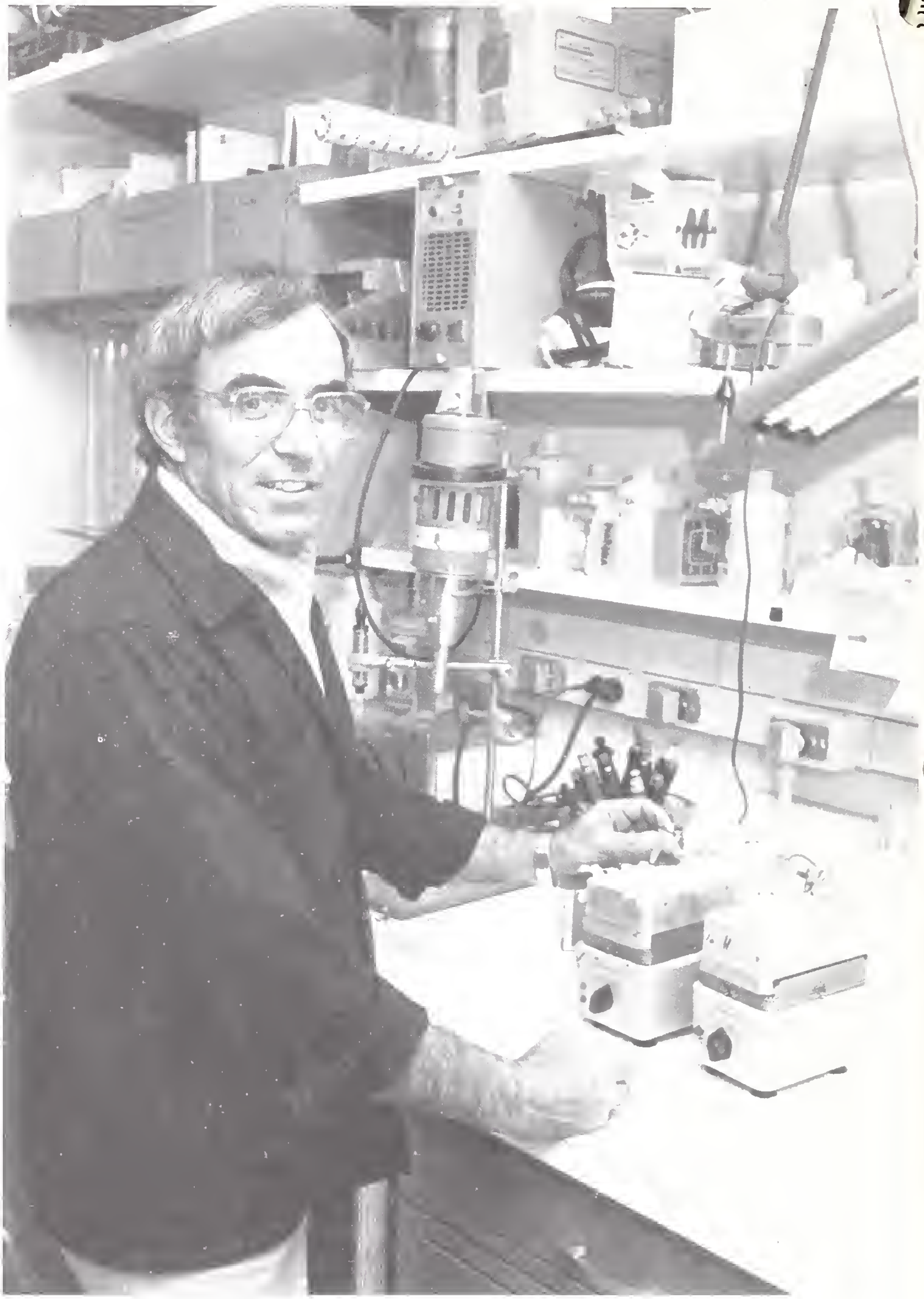
Editorial Staff

Susan Gerhold
Acting Editor
Sandra D. Levine
Assistant Editor
Maureen Napper
Editorial Assistant

NIH News Branch
Building 31, Room 2B-10
Bethesda, Maryland 20205
Phone:
(301) 496-2535

Biological Clock for Controlling Melatonin Production Identified



Dr. David Klein of NICHD investigates the 24-hour rhythm production of the hormone melatonin which appears to regulate seasonal reproduction cycles in some mammals.

NIH PHOTO 1807

Susan Johnson

A complex system in the brain that controls a 24-hour rhythm in the production of a hormone called melatonin has been demonstrated in the monkey, making it likely the same system that operates in humans. This finding was reported recently by a team of scientists from the National Institute of Child Health and Human Development (NICHD) and the National Institute of Mental Health (NIMH).

According to Dr. David Klein, the NICHD neurobiologist who headed the research team, melatonin production follows a pattern that is repeated every 24 hours, and this 24-hour rhythm is coordinated with environmental lighting, not with activity. In all mammals, regardless of whether they are active during the day or during the night, melatonin levels are low in the daytime and rise 500 to 2,000 percent at night.

Although melatonin is made in the pineal gland of the brain in all vertebrates, its production is controlled by a biological clock located elsewhere in the brain—within the cells of the suprachiasmatic nuclei. These are two small clusters of cells in the hypothalamus, an area of the brain that controls many involuntary body functions. The nuclei were named because of their position above the optic chiasm, the place where the optic nerves cross. A neural circuit connects these nuclei with the pineal gland.

The suprachiasmatic nuclei were identified in the brains of humans and other mammals many years ago, but their function was a mystery. Dr. Klein's studies—first in rats and now in monkeys—have shown that the nuclei turn melatonin production in the pineal gland on and off. Other researchers have found that the nuclei also regulate some other 24-hour body rhythms.

The brain clock controlling melatonin production is not highly accurate, Dr. Klein has found. In fact, it can be off by up to 30 minutes a day. Nature solves this problem by resetting the clock each day. The cues that reset the clock come from environmental lighting. The suprachiasmatic nuclei "learn" it is dark or light through electrical signals that travel from the eye to the optic chiasm, where they enter the nuclei.

Besides resetting the clock for melatonin production, the pathway from the eye to the suprachiasmatic nuclei serves a second important function. The signals it sends in response to light turn off communication between the nuclei and the pineal gland.

As a result, light can prevent or reverse the rise in melatonin that normally occurs at night. Research in laboratory animals has shown that melatonin levels remain low when animals are exposed to constant light. Similarly, when the lights are turned on after animals have been kept in darkness, a rapid drop in melatonin to daytime levels occurs.

Dr. Klein points out that although melatonin production in humans is coordinated with the light-dark cycle, much brighter light is required to turn off melatonin production in humans than in other mammals. Normal artificial indoor lighting does not affect melatonin production in humans.

Most of what is known about the system that controls the rhythmic production of melatonin, called the melatonin rhythm-generating system, came from Dr. Klein's studies in rats. The NICHD and NIMH team recently focused attention on the monkey, because of structural similarities between the monkey brain and the human brain.

They were encouraged to find that the rhythm and photic regulation of melatonin were the same in the monkey as in the rat. Their finding that the suprachiasmatic nuclei are involved in turning melatonin production on and off in the monkey strongly suggests that the nuclei have a similar function in humans, according to Dr. Klein.

The role of the melatonin rhythm in humans is not yet known, but it seems to be involved in regulating reproduction in many other mammals. In some animals that breed only during the warmer months of the year, the melatonin rhythm seems to be involved in turning reproduction on and off in response to seasonal changes in the length of the day.

Studies in hamsters, a seasonally breeding species, have shown that high levels of melatonin can inhibit reproduction if they occur at certain times of the day. This finding indicates there is a 24-hour rhythm in the sensitivity of the reproduction system to melatonin.

Dr. Klein speculates that during the fall and winter, when melatonin levels remain high longer because nights are longer, there is an overlap between periods of high melatonin production and periods of sensitivity to melatonin.

Unlike most mammals, humans do not have seasonal reproduction cycles. Dr. Klein believes, however, that melatonin could inhibit reproduction in humans, and that some infertility problems may involve melatonin. When scientists find a structure or chemical that has been preserved during evolution, they suspect it has a fundamental importance and that it functions similarly in many animals.

Dr. Klein predicts that within the next few years major breakthroughs will occur in understanding the function and importance of the melatonin rhythm-generating system in humans. ■

Researchers Assess Value of Plasmapheresis in Treating Lupus Nephritis Patients

Jim Fordham

The National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (NIADDK) is supporting a new nationwide multi-center study to determine the value of using plasmapheresis, a blood separating process, to treat patients who have severe lupus nephritis.

Plasmapheresis is a technique of removing the plasma from a patient's blood, and replacing it with plasma or a plasma substitute that does not contain the destructive antibodies found in lupus.

Systemic lupus erythematosus, a serious chronic inflammatory disease of the connective tissue, affects approximately 500,000 people in the United States, primarily young women. Lupus nephritis refers to the form of the disease that involves inflammation of the kidneys.

According to Dr. Nancy B. Cummings, NIADDK's Associate Director for Kidney, Urologic, and Hematologic Diseases, the purpose of this study is to determine whether plasmapheresis has a beneficial effect on patients with lupus nephritis, and to provide a mechanism to develop a standardized definition of lupus nephritis and guidelines for management.

In the past few years, several reports have appeared describing possible beneficial effects of plasmapheresis in the management of patients with severe lupus nephritis. After a pilot study, NIADDK grantee Dr. Edmund J. Lewis and his associates at Rush-Presbyterian-St. Luke's Medical Center in Chicago reported that plasmapheresis removed a large amount of damaging "immune complexes" (abnormal components) from the blood of patients. Scientists think that kidney and other manifestations of lupus may be directly related to injury caused by the presence of these abnormal components in patients' blood plasma.

The cost of plasmapheresis is high—about $10,000 for each patient's course of therapy. At present, only a small number of patients have been given this treatment. Reports of the success of plasmapheresis have prompted more widespread use of this therapy in conjunction with drug treatment, even though its effectiveness has not been proven.

In response to the need to better understand this new therapy, NIADDK awarded a grant to Dr. Lewis to conduct a controlled prospective study of the effects of plasmapheresis on patients with severe forms of lupus nephritis. As principal investigator for the study, Dr. Lewis will direct scientists who will study a total of 200 patients for up to five years at 16 centers throughout the country. Patients enrolled in the study will be randomly assigned to a group receiving either prednisone and cyclophosphamide, or plasmapheresis in conjunction with prednisone and cyclophosphamide. Prednisone is a steroid usually used to treat lupus nephritis. Cyclophosphamide, a drug that has a toxic effect on body cells, is often used in an effort to preserve functioning of the kidneys by immunosuppression.

Treatment is continuously monitored and, after an initial period, modified according to the patient's response. The goal of the scientists is to place patients on long-term, decreasing doses of prednisone after initial intensive therapy. Patients are evaluated through various tests using blood and uring samples. If the patient's kidney function does not improve, or if it deteriorates, physicians will continue the intensive administration of prednisone, cyclophosphamide, or plasmapheresis. Researchers will compare differences in occurrence and severity of medical complications and in survival between the two groups of subjects.

Scientists hope that this carefully controlled study will provide valuable information about the effectiveness and feasibility of plasmapheresis as a treatment for lupus nephritis, and the relationships between various tissue abnormalities and responses of patients to therapy. The study should also provide information on the mechanism of action of plasmapheresis in lupus nephritis, if the treatment is found to be beneficial.

One of the first manifestations of lupus, a major rheumatic disease, is arthritis, or joint inflammation. Substantial research on lupus is also conducted by NIADDK's Arthritis and Rheumatism Branch, and numerous investigations on lupus are supported by the Institute's Division of Arthritis, Musculoskeletal and Skin Diseases at research centers throughout the United States. ■

Neuro-PET Scanner Created by NIH Staff

Ray Fleming

The National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) has created a vastly improved positron emission tomography scanner called Neuro-PET because of its special ability to produce clear, precise pictures of the brain.

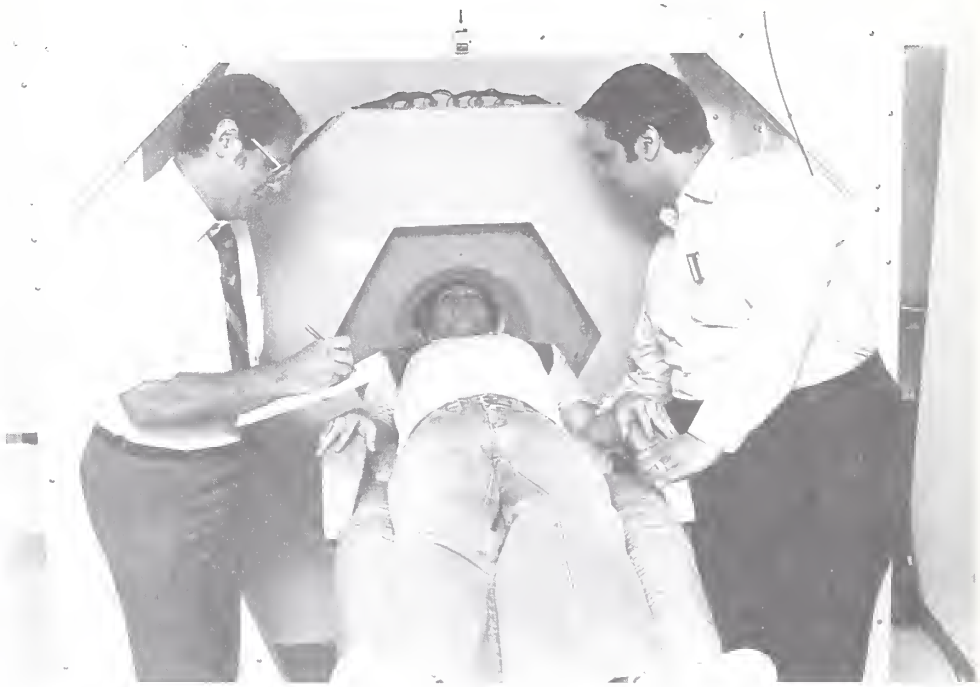
According to Neuro-PET's creators, Drs. Giovanni Di Chiro, Rodney Brooks, and Victor Sank, the new scanner's most distinctive assets are increased resolution and sensitivity which are essential in studying neurological and communicative disorders closely.

Neuro-PET will allow neuroscientists to probe more deeply into the brain to recognize and outline neurological structures that are not easily identifiable with currently available PET scanners.

Neuro-PET's increased resolving power should determine more precisely the location of the pathological involvement in such neuropsychiatric conditions as Alzheimer's disease, and in movement disorders, certain forms of epilepsy, and amyotrophic lateral sclerosis. It should also improve the assessment of degree of malignancy in primary brain tumors. This power may also make it possible to study the upper part of the spinal cord, an area of the central nervous system which has yet to be investigated by PET technology.

The Neuro-PET project began nearly four years ago, when the scientists from NINCDS's Neuroradiology and Computed Tomography Section confronted problems with standard PET scanners such as improperly focused pictures from low-sensitivity scanners, and resolving power that was insufficient to distinguish certain structures in the brain.

The quality of PET images depends largely on the efficient detection of gamma rays emitted in coincident pairs from the patient's brain—rays indirectly produced by decaying radioisotopes which are injected into the blood stream and accumulate in brain tissue. To get sharper images, the NINCDS team decided to concentrate on improving the usual array of crystal gamma-ray detectors used in PET scanners.



Drs. Victor Sank (left) and Rodney Brooks of NINCDS determine the position of the Neuro-PET scanner.

During an actual scan, a patient's eyes and ears are covered to minimize sensory information received by the brain.

NIH PHOTO 1808

After testing detectors of different materials and sizes, the scientists identified the arrangement that produced the greatest resolution and sensitivity: four rings of closely packed crystal detectors, each ring positioned directly behind another. In Neuro-PET, the large sodium iodide crystal detectors of standard PET scanners have been replaced with small, redesigned detectors made of bismuth germanate crystals.

Neuro-PET relies on 512 crystal detectors, many more than standard PET scanners, packed tightly into the small-diameter rings. These features allow Neuro-PET to detect more gamma rays in the brain, thus producing more detailed brain scans.

Scientists from NIH's Biomedical Engineering and Instrumentation Branch (BEIB) designed a highly sophisticated and complex electronics package for Neuro-PET. The package can identify coincident pulses from two simultaneous gamma rays among 40,960 possible pairs of detectors, and can code the pulses into numerical data to be transmitted to the computer. The BEIB scientists also designed and built a gantry to support the rings of detectors, and a mechanical system for allowing the rings to move in a unique "wobble" pattern around the patient's head during scanning.

The gantry, movement mechanisms, and electronics are the work of BEIB's Walter S. Friauf, Stephen B. Leighton, and Horace

Cascio. Alan J. Talbert of NINCDS's Office of Biometry and Field Studies contributed to Neuro-PET's software.

When fully operational, Neuro-PET will produce seven simultaneous brain-slice views, a feature that will significantly affect two aspects of the imaging process: the speed of the examination, and the precision alignment of the seven tomograms in relation to each other which is particularly important in identifying brain structures to be studied.

The scanner's multislice capability and speed of operation allows a larger number of patients to be studied and more efficient use of the radiopharmaceuticals involved in scanning.

Neuro-PET has also been an economic success. In-house design and some in-house construction have saved hundreds of thousands of dollars over the costs of commercially available scanners with less resolution and sensitivity.

Neuro-PET's usefulness will increase when a cyclotron, now in the planning stages, is installed on the NIH campus. With such a facility nearby, scientists will have immediate access to the short-lived isotopes that Neuro-PET needs. Presently, isotopes used in brain scanning at NIH are obtained from cyclotrons at the Naval Research Laboratory in Washington, D.C., and at Brookhaven National Laboratory in New York. By the time the isotopes reach NIH, a significant amount of activity has been lost to decay. A nearby source would also allow NINCDS scientists to use more kinds of isotopes, including isotopes that are now prohibited from being transported on commercial carriers.

According to Dr. Di Chiro, once the cyclotron is installed, "we may be able to perform two or even three sequential studies on a particular patient during the same session. The brain might be examined, for example, at resting state; after visual, auditory, and somatosensory stimulation tests; and after mental and pharmacological provocative tests."

Already an epilepsy patient and two patients with brain tumors, previously studied with a standard PET scanner, have been examined by Neuro-PET. And although the Neuro-PET scans were carried out four hours after isotope injection when much radioactivity had been lost, the resulting images verified the new scanner's high resolution capabilities.

Testing continues. Dr. Brooks, the project director, believes that "PET is an area of research where instrumentation capability is very important. The development of Neuro-PET has provided NINCDS with a state-of-the-art scanner which assures us of a leading role in this field." ■

Dr. Sank (center) adjusts the position of the Neuro-PET, while Dr. Brooks checks electronic settings.

NIH PHOTO 1809

Aerobic Exercises Produce Health Benefits for Kidney Patients

Jim Fordham

Aerobic exercise produces important health benefits for many patients with kidney failure, whose lives are dependent on dialysis to cleanse their blood, according to recent studies supported by the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (NIADDK).

More than 60,000 people in the U.S. are using artificial kidneys or are otherwise being dialysed for end-stage renal disease. Often these patients have numerous health problems that interfere with their normal activities and are predisposed to heart disease and stroke.

NIADDK grantees have found that an aerobics program can lower blood pressure, reduce anemia, and correct abnormalities in lipid (fat) and carbohydrate metabolism. They also noted that their patients had an increased capacity to do physical work and usually had a significant reduction in anxiety and depression, as determined by psychological testing.

Dr. Andrew Goldberg and associates at Washington University in St. Louis conducted studies which show that aerobic (endurance) exercises performed regularly result in a progressive lowering of serum triglycerides in patients whose blood fat levels are too high.

High blood pressure and abnormal lipid and carbohydrate metabolism are major risk factors for coronary artery disease. The advantages of reducing these risk factors are especially important for patients on dialysis, because studies show that these patients die from coronary heart disease more frequently than people without kidney disease.

The investigators also found that concentrations of insulin in the blood are reduced by aerobic exercise, a potentially desirable change from a diabetes-like metabolic condition that can occur in some kidney failure patients.

While more research is necessary to determine fully the benefits of exercise for patients on kidney dialysis, evidence so far indicates that it can prolong their survival and enhance the quality of their lives.■

Glycoproteins Affect Spread of Tumor Cells in Mice

Hilah Thomas

Scientists at the National Institute of Dental Research (NIDR), and the National Cancer Institute (NCI), report that a specific molecule (laminin), which mediates the attachment of certain cells to their surrounding extra-cellular collagenous matrix, probably plays an important part in the ability of tumor cells to spread to other sites in the body.

According to the June 1982 issue of *Cancer Research*, Drs. Victor Terranova and George Martin of NIDR, and Lance Liotta and Raimondo Russo of NCI, have shown that certain metastatic tumor cells use laminin to attach to surfaces coated with the type IV collagen characteristic of basement membrane (the noncellular layer separating epithelial (skin and mucous membrane) and endothelial (lining blood vessels) cells from connective tissues).

Laminin is one of a group of large, complex, glycoproteins which mediate the attachment of specific cell types to the genetically distinct types of collagen. Other examples of attachment glycoproteins are chondronectin which is associated specifically with cartilage, and fibronectin which links a variety of cells to types I and III collagen.

Scientists believe that these linking glycoproteins are important for normal morphological differentiation and development of organs and for repair of damaged tissue. They speculate that certain disease conditions lead to abnormalities in the relationships between cells and the extra-cellular matrix. Some cultured cells which have been transformed and become tumor-forming, either stop producing laminin and fibronectin or lose the ability to bind to the glycoprotein. Not all transformed cells, however, appear to lose the ability to bind to laminin. On the contrary, the ability of tumor cells to bind to basement membrane may determine their ability to spread.

When a malignant tumor spreads throughout the body, some of its cells leave the primary tumor and enter the circulation. Some of the cells traveling in the circulation come to rest in the small vessels of an organ. These arrested tumor cells then penetrate the vessel wall and start a secondary tumor colony. This process of metastasis formation is very complex, and one important initial step is the attachment of the tumor cells to the subendothelial basement membrane of the vessel wall. Consequently, the investigators decided to study the ability of certain metastatic and nonmetastatic tumor cells to attach to the type IV collagen of basement membrane. Adding fibronectin to the metastatic cell cultures did not increase their attachment, nor did addi[illegible] laminin to the nonmetastatic cells. Ho[illegible]ever, pretreating metastatic cells with antibody to laminin sharply reduced the attachment of all lines of metastatic cells tested.

In other studies, cells from two metastatic mouse tumors were divided into two populations: those which attached to type IV collagen by means of laminin, and those which did not attach. Although equally viable, these populations differed in their metastatic potential. After radioactively labeling the cells in culture, the scientists injected equal numbers of each population intravenously into mice. The cells that were unable to attach to laminin were cleared from the lungs of the mice faster than those able to attach. Post mortem examinations of the animals' lungs made 25 days after injection showed that metastasis developed more frequently from the cells capable of attaching to laminin than from the unattached ones. However, if the scientists pretreated either the parent population of cells or the subpopulation of cells able to bind to type IV collagen with antibody to laminin, the treated cells formed very few lung metastases in comparison with the untreated ones.

This evidence suggests that the metastatic potential of mouse tumors may depend in part on the ability of the tumor cells to use laminin for attaching to the type IV collagen found in basement membranes, particularly in blood vessels. ■

Mayo Clinic Researchers Release Data on Pityriasis Rosea

Prepared by the American Academy of Dermatology

A ten-year epidemiologic study conducted by dermatologists in Rochester, Minnesota, may have shed some light on the causes of pityriasis rosea—a skin disease of unknown origin, characterized by pale red patches with fawn-colored centers that appear on the trunk, neck and extremities.

As many as 40,000 people contract pityriasis rosea each year in the United States, according to researchers at the Mayo Clinic.

In the ten-year study, dermatologists supported in part by the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (NIADDK), examined 939 patients with pityriasis rosea who were living in the Rochester area. The study determined that the disease was most prevalent among women and younger people between the ages of 10 and 35. In addition, the study revealed that the greatest number of cases appeared during the cold winter months and the least number of cases were reported in the warmer summer months.

Since pityriasis rosea was first identified in the 18th century, its cause has remained elusive. Some of the possible causes considered include viral infection, allergies or contact reactions, stress or mental illness, or physical trauma. In some of the cases in Rochester, the onset of the disease appeared with other skin conditions or pregnancy.

The Mayo Clinic researchers are currently investigating any risk factors linked to the disease that may help provide clues to its cause. Several investigators say that one such link may center around the presence of infection in the patient's body. Indeed, they found that one-fifth of the people examined in the study had some type of acute infection within three months prior to the diagnosis of pityriasis rosea, many involving the upper respiratory tract. The investigators also noted that the low rate of recurrence and the incidence of more than one person in a household developing the disease at the same time tend to support the theory that this condition is related to infection.

Previous discussion on the causes of pityriasis rosea has brought up the possibility that the inherited characteristics of an individual's immune system (which works to fight off infection and can also be triggered by allergies, drugs or stress) may be the key to understanding the mechanism of the disease. When it is called into action by one of these conditions, there may be an immunological-related reaction that appears on the skin in the form of pityriasis rosea. The itching, inflammation and the raised red patches associated with pityriasis rosea usually disappear within six to eight weeks.

Results were published in the July issue of the *Journal of the American Academy of Dermatology*. ■

Cell Mechanism May Someday Provide Selective Pathways to Fighting Cancer Cells

[illegible]rrell Wolfson

Through new approaches that combine microscopy and biochemistry, Drs. Ira Pastan and Mark Willingham of the Laboratory of Molecular Biology at the National Cancer Institute (NCI) have been able to identify a number of separate steps in the process called "receptor-mediated endocytosis."

Endocytosis is a mechanism in which large, needed molecules from the environment must surround and enter the cell because they are too large to pass directly through the cell membrane or to be transported in by "carrier" molecules. Although this process, which helps regulate cell response to growth factors, takes place in all cell types, the steps in the pathway have not been understood until recently.

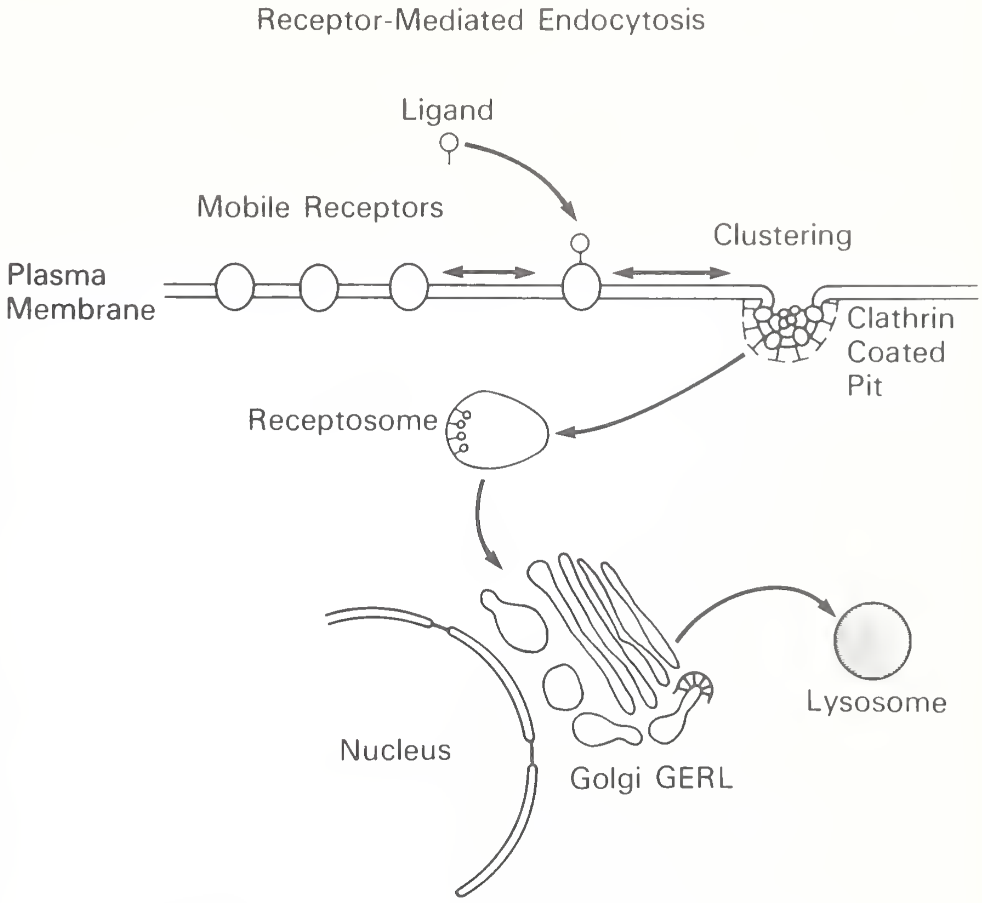
According to Dr. Willingham, "Knowledge of this pathway may some day help scientists design anticancer drugs that can be taken up selectively by cancer cells. One even more basic research thread is that normal cells, in tissue culture, can be made to mimic transformed or cancer cells by overwhelming them with growth factors. These growth factors bind to the cell via surface receptors and some go on to enter the cell through this pathway. Studying this process may unearth information on the process of transformation."

The steps include binding of ligands (molecules that bind to specific receptors) to receptors that are diffusely distributed on the cell surface, and movement of the ligand-receptor complexes to bristle-coated pits and internalization within cell structures the NCI investigators have named receptosomes (receptor-bodies).

Hundreds of thousands of receptors are embedded in each fibroblast membrane. Usually large proteins, they include receptors specific for growth factors, hormones, lipoproteins, lysosomal enzymes, and even toxins and viruses. Most are randomly distributed and constantly in motion along the fluid cell surface.

NCI investigators have been able to trace the paths of fluorescently-labeled ligands using a technique they developed called video intensification microscopy. This technique substitutes an intensifier television camera for the eyepiece of a fluorescence microscope, enabling hormones and other ligand-receptor (L-R) complexes to be seen on the fluid cell surface.

Like tiny basins along the cell surface, bristle-coated pits, about one thousand per cell, cover only one to two percent of the total cell surface area. The pits are stable elements of the membrane and different types of L-R complexes can cluster together in a single coated pit. After clustering, the specialized transport structures called receptosomes take shape.



Receptor-mediated endocytosis is the mechanism by which needed molecules from the environment enter the cell through the movement of ligand-receptor complexes to bristle-coated pits and internalization within receptosomes.

NIH PHOTO 1810

Other researchers had previously noted the presence of recently ingested ligands in a variety of intracellular structures. Drs. Pastan and Willingham showed that the first intracellular structure a ligand enters is a receptosome. Further, they observed receptosome movement in living cells, and verified that they contained substances that entered the cell by way of surface receptors. Receptosomes are not "coated" like pits, and appear to form from an infolding of a portion of the membrane adjacent to a pit. A new receptosome forms as often as every 20 seconds.

The L-R complexes that have clustered in a pit slide into the receptosome, which shuttles them to the cell interior. Traveling in small, gliding movements, receptosomes can move halfway across a cell in a matter of seconds. Ultimately, they bring their contents to the cell interior.

Endocytosis that does not rely on receptors often delivers molecules directly to cell lysosomes, minute structures filled with enzymes that rapidly degrade the molecules. In contrast, the receptosome delivers the ligand, and sometimes the receptor, to the Golgi-GERL system near the nucleus. The Golgi apparatus is a structure that directs the flow of intracellular proteins, and may be responsible for recycling the receptors back to the surface. The Golgi also processes ligands, extracts proteins, and directs them to various sites in the cell. The GERL (Golgi endoplasmic reticulum lysosome) complex is a part of the Golgi that has some role in compartmentalizing the ligands.

After processing, the ligands are delivered to nearby lysosomes, where the molecules are finally broken down. According to Dr. Willingham, "With low-density lipoprotein (LDL), for example, the lysosome degrades the LDL protein shell that surrounds cholesterol. It then releases cholesterol, which somehow makes its way into the cytoplasm of the cell." Meanwhile, the intact LDL receptor finds its way back to the cell surface completing the internalization cycle that carries receptor-selected substances from the cell surface to the cell interior.

A review of the most recent findings on this receptosome pathway appeared in the October 1981 issue of *Science*. ■

Grappling with Grip: Progress and Problems in Prosthesis Research

Ray Fleming

Dr. F. Terry Hambrecht of the Neural Prosthesis Program at the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) is involved in developing an electrical system that will return hand control to patients with paralyzed muscles.

According to Dr. Hambrecht, "A person with a spinal cord injured at a high level has many disabilities, but one of the most devastating is the loss of hand grasp and sensation. We are attempting to restore this lost function by electrically stimulating the paralyzed muscles." Many spinal cord injury patients, for instance, are unable to perform such simple tasks as answering a phone or drinking from a cup, and their inability to feel and grasp objects is a major obstacle to independent living.

The grasping ability that scientists have been trying to restore is lost when, through traumatic injury to the spinal cord, the flow of nerve-borne messages from the brain to the hand is interrupted. But by direct electrical stimulation of the undamaged motor neurons attached to the hand muscles, the spinal cord can be partially bypassed and a modified grip restored. Building on this concept, called functional neuromuscular stimulation (FNS), Dr. Hambrecht, working with scientists at Case Western Reserve University in Ohio and the University of Utah Research Institute, is directing development of a three-part prosthetic device that will permit limited hand movement.

The device features small electrodes implanted with a hypodermic needle into a paralyzed patient's thumb and forefinger muscles. These electrodes are then connected to a computer-controlled stimulator, which provides the power to excite the muscles in a certain sequence and at a particular level of strength. Patients wear an electrical device called a position transducer near the shoulders, one of the few parts of the body that usually remains mobile after severe spinal cord injury of the neck. This transducer translates the patient's deliberate shoulder movements into commands to the stimulator.

Dr. Hunter Peckham at Case Western Reserve University has tested this application of FNS in patients, with promising results. Once the hand muscles have been electrically exercised for several weeks to reverse atrophy, stimulation of the implanted electrodes will allow the trained patient to grasp objects and perform other tasks.

Two types of grip are possible with the prosthetic system: lateral pinch grasp, which allows the patient to pick up small objects, and palmar-prehension grasp, in which the thumb and fingers are used to pick up larger objects. The computer-controlled stimulator "provides smooth, functional grasp with a minimum of muscle fatigue," and can "change the activity patterns of up to nine different muscles for positioning the fingers" according to Dr. Hambrecht.

The system reacts quickly to commands and, most importantly, is under the patient's control, helping to restore the independence and self-esteem sometimes lost in a paralytic disability.

The hand prosthesis is still in the experimental stage and several problems remain. The implanted electrodes tend to move slightly when muscles contract, which can change the strength of the muscle's reaction to stimulation. In addition, the electrodes must be connected to the stimulator every day, a time-consuming task impossible for a quadriplegic individual to manage alone. But Dr. Hambrecht believes that a totally implantable stimulator and electrode system now being designed should reduce these problems.

A second area of concern is feedback: allowing the patient to feel where the fingers are placed and how hard they are grasping. Since the injured spinal cord can no longer relay messages back to the brain, a way must be found to send muscle strength and position information back to the stimulator, where adjustments can be made. To solve this problem, NINCDS contractors at the University of Utah Research Institute are developing sensors which, when worn as part of a transparent glove, will generate signals to be used by the stimulator or monitored directly by the patient. The patient could receive such signals either as sound or vibrations directed to the skin above the level of sensory damage.

More patient control over the hand prosthesis is another of Dr. Hambrecht's goals. Because of the limited range of motion in the shoulder area, the movement-activated transducer has only a small number of possible commands which it can relay to the stimulator. A voice-activated transducer has been proposed to solve this problem, but some paralyzed patients may be reluctant to call attention to their disability by using vocal demands.

New computer programs will be needed to expand the number of muscle commands that the stimulator can now pass on to the electrodes. To give the patient more control, the computer must provide more flexibility in the order of muscle activation and in the strength of muscle response. Patient control might also be improved by the implantation of more electrodes, a possibility that is now being investigated.

Dr. Hambrecht believes that the future of the prosthetic system will move beyond hand rehabilitation, for the FNS principles involved can also be applied to the wrist, elbow, and legs. He is hopeful that as the techniques are perfected, they can also be used in the rehabilitation of the arms and hands of stroke patients.

Years ago, scientists used FNS principles to develop a device for controlling dangerously irregular heartbeats. Today, the cardiac pacemaker is helping its many recipients to lead normal, productive lives. For Dr. Hambrecht and others in neural prosthesis research, such a success story is well on its way to being repeated. ■

Environmental Factors Examined in Causing Facial/Cranial Malformations

Tom Hawkins

Approximately one in every 500 babies born in the U.S., 10,000 infants each year, suffer from cranial or facial malformations, usually cleft lip or palate.

Robert M. Pratt, Ph.D., a research chemist at the National Institute of Environmental Health Sciences (NIEHS), is among the nation's leading researchers on the biochemical mechanisms of birth malformations, especially cranial-facial defects. He points out that these malformations most often occur when the mother and embryo have a genetic predisposition, and when certain environmental factors such as chemical or drug exposure are present.

According to Dr. Pratt, "The evidence suggests that clefting of the palate is nearly always multifactorial. If we can learn more about the basic mechanisms of normal and abnormal embryonic development, and identify those environmental agents involved, we think it may be possible to protect many vulnerable mothers from the environmental factors that trigger these defects in the developing embryo."

Pratt and his research team use various mouse strains as a model for the biochemical and morphological sequence of events that leads to clefting in humans. Various drugs and environmental chemicals are administered to pregnant mice to ascertain the types of induced malformations. Culture of tissues and cells from the embryo is the major experimental technique used in the laboratory. Primary cultures of mouse palatal mesenchymal (connecting tissue) as well as epithelial cells are used to study the biochemical mechanisms by which various chemicals cause cleft palate. In addition, human cell cultures (established cell lines) grown from tissues obtained from abortuses or during corrective surgery of cranial-facial malformations provide an invaluable approach to understanding basic mechanisms of growth control and how defects are triggered.

In order to study the development of the embryo inside the mother, mouse embryos are grown in glassware in a special nutrient "broth".

The embryos grow for about 48 hours and show normal patterns of development as well as the impact of exposures to environmental chemicals. Using such methods the researchers in Dr. Pratt's laboratory are learning about some of the mechanisms of facial and palatal clefting.

NIH PHOTO 1811

Hormones, potent chemical messengers in the body, each have specific receptors which receive their message and initiate their action. One chemical that causes cleft palate in mice is the potent tetrachlorodibenzo-p-dioxin (TCDD). According to Dr. Pratt, "Hormones, specifically glucocorticoids (cortisone for example) are essential in the normal development of the embryo and fetus. A certain level of glucocorticoid and its receptor is required, yet more than that level will cause cleft palate to occur. Like the synthetic chemical TCDD (a component of Agent Orange), other chemicals may behave in the embryo like hormones, binding to receptors and disrupting the normal balance of biochemical processes within the cell."

Epidermal growth factor (EGF) is a small protein that influences certain aspects of the developing embryo and fetus and apparently disappears at birth. Dr. Pratt and his coworkers have shown that the developing palatal mesenchymal and epithelial cells are highly dependent on the presence of EGF and it is likely that an embryonic type of EGF is produced by a number of embryonic tissues including the palate.

Another exciting direction in the lab's research is in the use of vitamins to reduce teratogen-induced clefting malformations in the mouse. Pregnant mice treated with vitamin B_6 show a markedly lower incidence of cortisone-induced clefting in their offspring. "This research and its possible clinical implications must be interpreted with great care," Dr. Pratt cautioned, "because too much of any chemical, even a vitamin or growth factor, may harm the development of the embryo and fetus."

Dr. Pratt's research efforts in the future will emphasize many of the currently available techniques in molecular biology, such as gene cloning, which will eventually lead to a better understanding of the intricate molecular mechanisms that insure normal embryonic and fetal development. ■

Scientists Note Glucagon Plays a Major Role in Diabetes

Pat Sheridan

Research in recent years has suggested that excessive amounts of the hormone glucagon contribute to elevated blood glucose levels in diabetes.

University of Arizona scientists Drs. David Johnson and Victor Hruby, under a grant from the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (NIADDK), recently reported development of a glucagon antagonist, THG, that blocks the action of glucagon and significantly lowers blood glucose levels in diabetic rats. This finding provides further evidence that the metabolic abnormalities that characterize diabetes may result in part from the effects of glucagon.

Studies in the late 1960's revealed elevated glucagon levels in virtually all forms of human and animal diabetes. Although glucagon, like insulin, is secreted by the pancreas, these hormones have very different effects—insulin lowers the blood sugar level, while glucagon raises it. Glucagon normally stimulates the liver to release stored glucose into the blood for energy during periods of fasting or extreme physical stress.

In diabetes, however, the usual suppressing effect that circulating blood glucose has on glucagon secretion is lost. This creates "double trouble" for diabetics—blood sugar is elevated, not only by a lack of insulin, but also by the extra glucose that glucagon is releasing from the liver. Both abnormalities must be corrected to achieve complete diabetic control.

Johnson and Hruby and their associates focused on the development of a glucagon inhibitor that would interfere with the hormone's action on target tissues. Their research produced THG, an analog of glucagon that blocks the liver's receptors for the hormone. This action prevents the binding of glucagon to its receptors, a process essential for triggering biological activity. When THG was administered to rats made diabetic with streptozotocin, their blood glucose levels decreased by up to 50 percent, indicating that glucagon action contributes to the blood glucose elevations in this animal model of diabetes.

The investigators suggest that the synthesis of this glucagon analog may serve as a prototype for the development of other hormone antagonists. Further studies will be necessary, however, to determine whether THG or other specific glucagon antagonists might be of benefit in the treatment of patients with diabetes. This report was published in the February issue of *Science*. ■

Medical Capsules

Sandra D. Levine

Medical Capsules is a new component that will be appearing in *News and Features*. It is designed to provide information in the form of brief capsule summaries about research being conducted at NIH or by its grantees, of current interest or potential future importance.

- A study sponsored by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) of the babies of over 7,000 women who worked until their 28th, 33rd or 37th weeks of pregnancy, revealed progressively lower birth weights and a greater incidence of placental infarcts the longer the mothers had stayed on-the-job. This did not necessarily mean, however, that the later development of these smaller babies would be affected. (See Naeye and Peters, *Pediatrics*, June 6, 1982, pp. 724-727).

- A major randomized study under the auspices of the National Heart, Lung, and Blood Institute (NHLBI) to evaluate the use of streptokinase in opening blocked coronary arteries is beginning in three New York City centers. The enzyme streptokinase was approved by the FDA for intracoronary use in May. The study will take at least five years to complete, and plans are also underway for a larger 10-12 center study. (See *JAMA*, Aug. 20, 1982, p. 805).

- Scientists at the National Institute of Environmental Health Sciences (NIEHS) in North Carolina have investigated the unique role of the Clara cell, a particular kind of cell interspersed in the tissue of the lung and respiratory tract. The Clara cell, a relatively large cell with no fiber-like cilia characteristic of other lung cells, has been separated from other cells and grown in culture in the NIEHS Laboratory of Pharmacology. Using this technique, it has been determined that Clara cells have unusually high metabolic and enzymatic activity, and play an important role in interacting with environmental toxins. The study of Clara cells is one key part of developing a scientific understanding of normal respiratory function and pulmonary toxicology.

- Researchers at Johns Hopkins University, using data from a survey supported by the National Institute of Child Health and Human Development (NICHD), conducted a study to determine whether there is statistical evidence to support the contention that sex education in schools leads to increased sexual activity and unplanned pregnancy among teenagers. The analysis of responses from over 3,700 teenagers showed that those who have taken a sex education course are no more likely to be sexually active than those who have not, and indeed are less likely to become pregnant before marriage.

- Physicians in Boston, under grants from the National Institute of General Medical Sciences (GMS), are preparing to test in humans a new type of artificial skin for the treatment of burns. A simpler version is currently being used, but the new type is "seeded" with the patient's own skin-forming basal cells to produce an intact skin layer more rapidly, without rejection, and with less shrinkage of tissues. (See *Science*, Aug. 6, 1982, p. 522).

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News&Features from NIH

November 1982

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Are Some Women Really Affected by Their Menstrual Cycle? **p. 16**

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DRS and DCRT Team Develops New Electron Microscope **p. 12**

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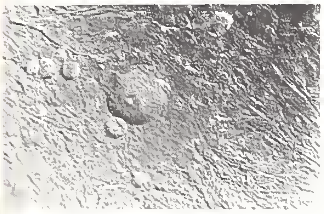
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

NINCDS has funded a Stroke Data Bank which compiled information on over 1,000 patients. Using the most sophisticated diagnostic techniques available, types and subtypes of strokes were defined. The information contained in the data bank will be used as an aid in the diagnosis and treatment of strokes and to further research advances into these disorders. See page....6



NIH scientists have developed a new electron microscope capable of chemical microanalysis of a specimen and correlation of this analysis with the specimen's microstructure. This highly sophisticated instrument is the result of collaboration among several scientific disciplines, involving physicists, engineers and computer scientists. See page....12

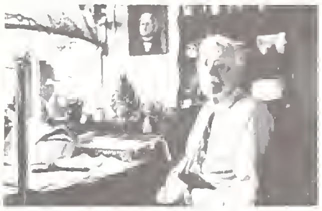
News&Features from NIH

November 1982

The toxic effects of anticancer drugs on bone marrow have made it necessary to limit doses of chemotherapy. Now, however, a procedure called autologous bone marrow transplantation may reduce these toxic effects. The technique involves removing a sample of the patient's own bone marrow cells before there has been any damage from anticancer drugs. The marrow cells are then frozen and returned to the patient at a later time. These cells then begin to grow into healthy bone marrow. Problems remain with the technique, but research is making advances so that patients may receive more extensive amounts of anticancer therapy. See page....14

Scientists at the University of New Mexico have found significantly larger amounts of the autoantibody known as rheumatoid factor present in the blood of people over the age of 70. Lymphocytes from the blood of elderly donors also showed abnormalities in the high amounts of rheumatoid factor production.

The immune system in old age often produces too many autoantibodies—substances that attack the body's own cells rather than foreign cells. See page....15



The editorial staff of *News and Features* wishes to clarify an ambiguous statement which appeared on page 2 of the October issue. The first sentence should have read: "A complex system in the brain that controls a 24-hour rhythm in the production of a hormone called melatonin has been demonstrated in the monkey, making it likely that the same system operates in humans."

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Editorial Staff

Susan Gerhold
Acting Editor
Sandra D. Levine
Assistant Editor
Maureen Napper
Editorial Assistant

NIH News Branch
Building 31, Room 2B-10
Bethesda, Maryland 20205

Phone:
(301) 496-2535

Nutritional and Educational Programs Now Being Applied in Several South American Countries

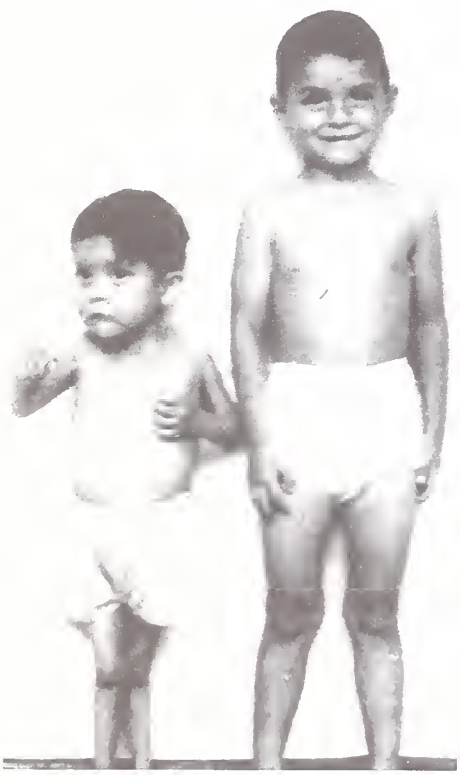
James Hadley

A national child nutrition and early education program, now in its sixth year of operation in Colombia, South America, is a direct result of U.S.-sponsored medical research in that country. These investigations are greatly increasing our knowledge of the effects of nutrition and early behavioral stimulation on intellectual development and are also improving the growth and health of Colombian children.

The studies, coupling nutritional supplements with behavioral stimulation, were initially funded by the Ford Foundation and later continued by the National Institute of Child Health and Human Development (NICHD). They were part of a coordinated series of studies designed to clarify the relationship between malnutrition, learning, and behavior, funded by NICHD throughout the Western Hemisphere.

Dr. Merrill S. Read, Chief of the Clinical Nutrition and Early Development Branch at NICHD, discusses the results of these and other research projects in a "state-of-the-art" report entitled "Malnutrition and Behavior." The paper, published in the fall issue of *Applied Research and Mental Retardation*, was the basis of Dr. Read's recent presentation at the University of North Carolina, Greensboro.

Guatemalan children are given a nutritional supplemental drink which, when coupled with behavioral stimulation, researchers say, can prevent and treat malnutrition. **NIH PHOTO 1752**


Both these boys are five years old, but the youngster on the left suffers from malnutrition. **NIH PHOTO 1751**

In general, Dr. Read said, research from two decades of study around the world has shown that malnutrition and social environmental factors may interact to influence the development of a child's central nervous system and also help shape behavior.

The two studies in Colombia were designed to explore the effectiveness of coupling behavioral and nutritional interventions to prevent and treat malnutrition. One project was designed to teach low-income mothers in Bogota, Colombia how to use household objects to encourage cognitive development in their infants while at the same time the program provided nutritional supplements. The other introduced a multi-faceted nutrition, health and educational intervention program over a three-year period. This program focused on three to five-year-old children in a preschool setting in Cali, Colombia.

Results indicate that unsupplemented infants became more irritable during testing sessions and reacted more strongly to aversive stimuli, than did supplemented infants. Both nutritional and behavioral stimulation significantly enhanced performance on standardized tests. The supplementation had a greater effect on motor functioning while educational intervention primarily affected linguistic development. Thus, the two interventions appear to influence behavior in different ways. The greatest changes were in those children receiving both interventions. However, even in those cases, the results from the double intervention were not equal to the test scores of middle and upper-class Colombian children.

Earlier animal and human studies on malnutrition and mental development examined severe malnutrition which, Dr. Read said, refers to severe restriction of protein or energy intake, or both, in early childhood. Malnutrition produces extremely stunted growth, water retention, skin sores and hair discoloration. If untreated, the condition can lead to death. Severe malnutrition is not restricted to poverty-stricken homes. Although ordinarily found in developing countries, it sometimes occurs in the United States cases of severe parental neglect.

Dr. Read said NICHD-supported research demonstrates that severe malnutrition in animals and humans during the fetal or infancy period, particularly if prolonged, may alter the structure and function of brain cells.

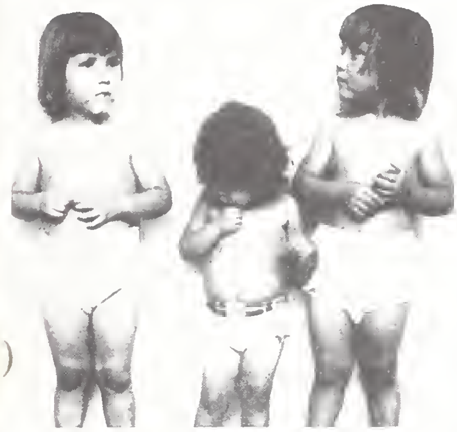
Compared to severe malnutrition, moderate malnutrition or undernutrition plagues a larger percentage of the world's children. This condition is caused by an inadequate diet where both the quantity and quality of food intake is restricted. Because of the deficient diet, the child grows at a much slower rate with low resistance to childhood diseases such as measles, diarrhea and pneumonia. Anemia, rickets and goiters can also result from malnutrition.

Environments providing little educational stimulation or emotional support may also impair a child's learning abilities, according to Dr. Read. For example, family disintegration may lead to a child's being neglected or abandoned. Such children often fail to respond adequately to the few stimuli available. This disrupts the developmental process and learning is hindered.

Usually the better nourished a child is, the better his social interaction with family, peers and teachers. In a well-nourished child, intellectual potential is more likely to be attained because the child is more responsive to the relevant stimuli available in the learning environment. The child interacts better in school and relates to others in a positive manner.

Changes in personality, emotion and behavior accompanying malnutrition may interfere with the interpersonal relationships necessary for learning. The malnourished child tends to be apathetic, disinterested, irritable and disruptive in class. Thus, the child may demand little from his environment, either nutritionally or in terms of social experiences.

Research indicates that better dietary management for pregnant women may improve the outcome of pregnancy, particularly in the area of infant birthweight. Nutritionally supplemented infants also show significant improvement in growth and small, but significant, improvements in performance on mental and behavioral tests, especially social and motor skills and alertness. They also are more active and demanding of parental attention.



These three children are all over two years old. The child in the middle suffers from malnutrition. The other two—twins—are developing normally. **NIH PHOTO 1753**

Follow-up studies on the Colombian children entering fourth grade showed significant differences between children having two or three intervention periods and those having fewer, according to Dr. Read. Test scores measuring the low-income children's problem-solving skills increased over a period of time.

The earlier and more prolonged interventions led to higher scholastic achievement by the time a child reached third grade and to fewer school failures compared to children with little or no intervention.

However, Dr. Read said, test scores involving language development declined during the school years for all children from low socio-economic environments. This deficit, he suggested, may be similar to the impairment in language development due to prenatal and infancy malnutrition reported in other studies; all low socio-economic status children were undernourished at the time the study began.

A similar follow-up study on Guatemalan children six to eight years old found that increased calorie supplementation from birth to four years of age predicted greater social responsiveness, more interest in the environment, higher physical activity levels, greater affective expression and improved attentional processes. On the other hand, low supplementation was associated with passivity, dependency on adults and anxious behavior. There was little or no effect on cognitive measures.

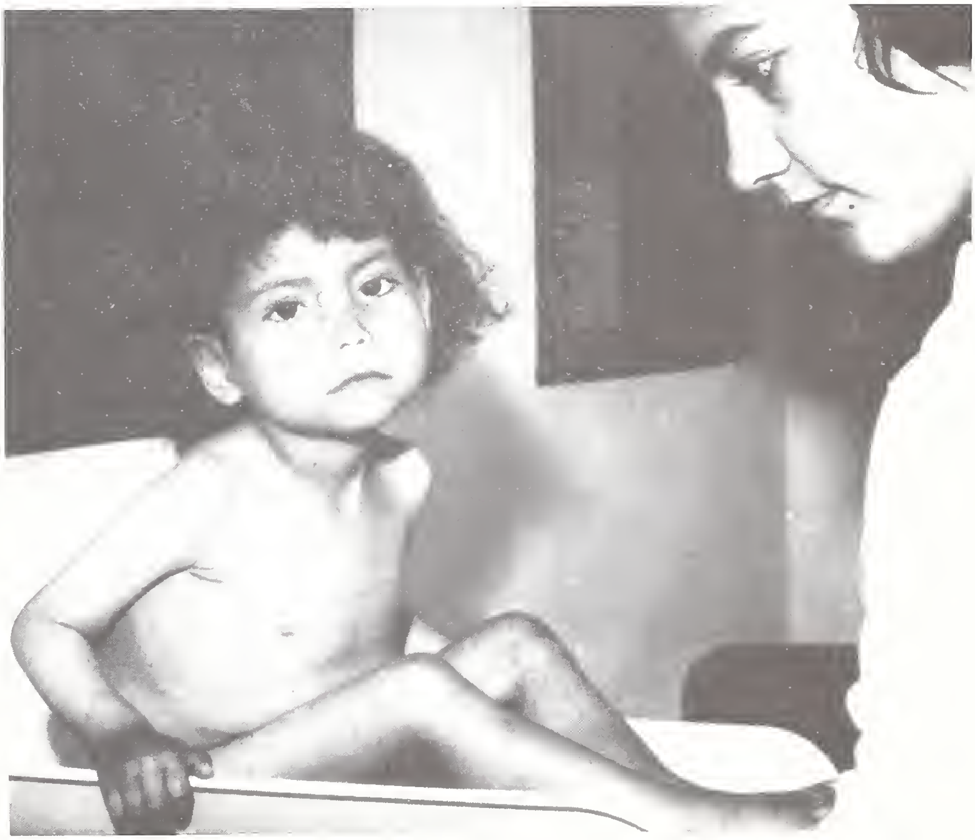
These researchers concluded that "the child attempts to adapt to the physiological stress of nutritional deficit by developing behaviors that remove or isolate him/her from the social and non-social environment. These behaviors inhibit the later development of appropriate patterns of social interaction."

A rather recent report in Mexico showed that despite similar IQ scores, one-third of the undernourished children did not pass the first year of school work, while none of the supplemented children failed. The supplemented children were more attentive in school, participated more in classroom activities and answered more questions from the teacher. The poorly nourished children spent more time daydreaming, crying or sleeping. As the day progressed, these characteristics became more pronounced and the children became more irritable.

Therefore, it is apparent, concludes Dr. Read, that moderate malnutrition in early life, including pregnancy, may adversely affect aspects of cognitive development in infancy, particularly in language development. These deficits do not appear to be permanent, however, especially when the environment is stimulating. Of greater long-term significance is the impact of malnutrition on physical activity and social behavior. Malnourished school-age children continue to have greater difficulty than supplemented children in attentiveness, independence, anxiety, achievement and participation in school activities. These characteristics hinder a child's success in a developing culture where change and creativity may be necessary hallmarks of success, he said.

Investigators will continue to make strides in understanding malnutrition and behavior, Dr. Read said. More data are needed, he added, to help "reorder our maternal, infant and child programs to maximize benefits for the mother and child and to help babies grow to be healthy adults." In order to target programs to those in the high risk category, he said, scientists must identify the specific characteristics of the mothers and children who will benefit most from nutritional supplements.

Research is also needed to help define the effects of zinc, iron and other nutrients on behavior and development, he emphasized. Environmental variables, such as food choice (infant formula or breastfeeding), polutants, poverty and drugs also should be investigated to see what role they play in malnutrition and in child development. ■



A child is weighed by a nurse as part of the nutrition and early development program in Bogota, Colombia. **NIH PHOTO 1754**

Scientists Isolate Gene Linked to Lesch-Nyhan Syndrome

Doris Parker

Some day in the not too distant future, doctors hope that they will be able to treat genetic diseases by replacing faulty genes with healthy ones. The DNA (or deoxyribonucleic acid) molecule that determines our heredity is beginning to yield its secrets. Genes, which are segments of DNA that carry the instructions necessary for the body's cells to grow and reproduce, are now being isolated, studied, and manipulated in the laboratory.

The recent cloning of an important human gene by Dr. Theodore Friedmann, a grantee of the National Institute of General Medical Sciences (NIGMS) working at the University of California at San Diego, is a valuable step forward in this developing technology. The newly cloned gene codes for an enzyme, hypoxanthine guanine phosphoribosyl transferase (HPRT). A deficiency of HPRT in humans causes a rare but devastating form of cerebral palsy called Lesch-Nyhan syndrome and is also implicated in some forms of gouty arthritis.

Children with Lesch-Nyhan syndrome are often severely retarded and have such difficulty controlling aggressive and destructive behavior that they may compulsively bite off their own lips and fingers. The syndrome is a male disorder, since the HPRT gene is located on the X chromosome. Females, who have two X chromosomes (males have one X and one Y), are protected from the disorder by the tremendous odds against having two defective X chromosomes in the same individual. If a woman carries an X chromosome defective for the HPRT gene, though, she may pass on the defective gene. In that case, each male child will have a 50% chance of being affected by Lesch-Nyhan syndrome.

Victims of Lesch-Nyhan syndrome, such as this boy, may compulsively bite off their own lips and fingers.
NIH PHOTO 1755

When the HPRT gene is defective, cells cannot properly recycle certain chemicals, which accumulate and damage nerve cells and other tissues. Some HPRT defects cause a form of gouty arthritis in which uric acid crystals accumulate in the joints. Thus, study of the HPRT gene may shed some light on the nature of metabolic defects associated with gout. It is not clear why some HPRT gene defects cause relatively mild disorders and other defects cause severe diseases.

Although it is still a long step to finding some way to get the cells of persons with genetic diseases like Lesch-Nyhan syndrome to make or accept enough healthy genes to correct their disorders, the way is opening for many important studies. According to Dr. Friedmann, the HPRT gene may well become a model system for the study of hereditary diseases.

Certain unique features of the HPRT gene make it a particularly promising research tool. Scientists can use it to study how genes affect neurological function and behavior. Also, it is one of the few genes which have been cloned that are located on the important X chromosome. Many disorders in addition to Lesch-Nyhan syndrome are linked to the X chromosome. These include color blindness, hemophilia, and some forms of muscular dystrophy and mental retardation. Research with the HPRT gene will help scientists understand the workings of this chromosome.

Although Dr. Friedmann and his coworkers were the first to clone the human HPRT gene, many scientists have been working in this area. Recently, doctors at Baylor College of Medicine cloned a mouse gene which is similar to the human gene and which codes for HPRT in that animal.

Dr. Friedmann first became interested in HPRT in 1967, when Dr. J. Edwin Seegmiller, then at NIH, first demonstrated that HPRT deficiency was associated with Lesch-Nyhan syndrome. Techniques for picking specific gene segments out of the DNA molecule were not available then. Two and a half years ago Dr. Friedmann returned to HPRT research, using methods, including recombinant DNA techniques, developed in the intervening years. Workng with him in this effort were Dr. Douglas Jolly, Dr. H. Uli Bernard, and Ms. Abby Esty.

By a process called transfection, the researchers succeeded in getting mouse cells lacking HPRT to take up human DNA, including functioning human HPRT genes. A solution called HAT medium, which allows only cells with normal HPRT function to grow, was used to eliminate cells not making HPRT. Then through several subsequent rounds of transfection, using DNA from the surviving cells, the investigators removed most of the human DNA not coding for HPRT.

The trick, though, was to extract the human HPRT gene from the mouse DNA. They attempted to do this using restriction enzymes—a chemical means of cutting DNA—and plasmids to carry the cut pieces into bacteria. If the HPRT was present on a piece of DNA, the bacteria into which it was carried should make the enzyme.

But the scientists discovered that the HPRT gene was very large, and the enzymes cut into the gene itself so that no functioning piece could be isolated. So they decided to search the transfected mouse DNA for some identifiably human genetic fragment that might be attached to the human HPRT gene and used to retrieve the gene.

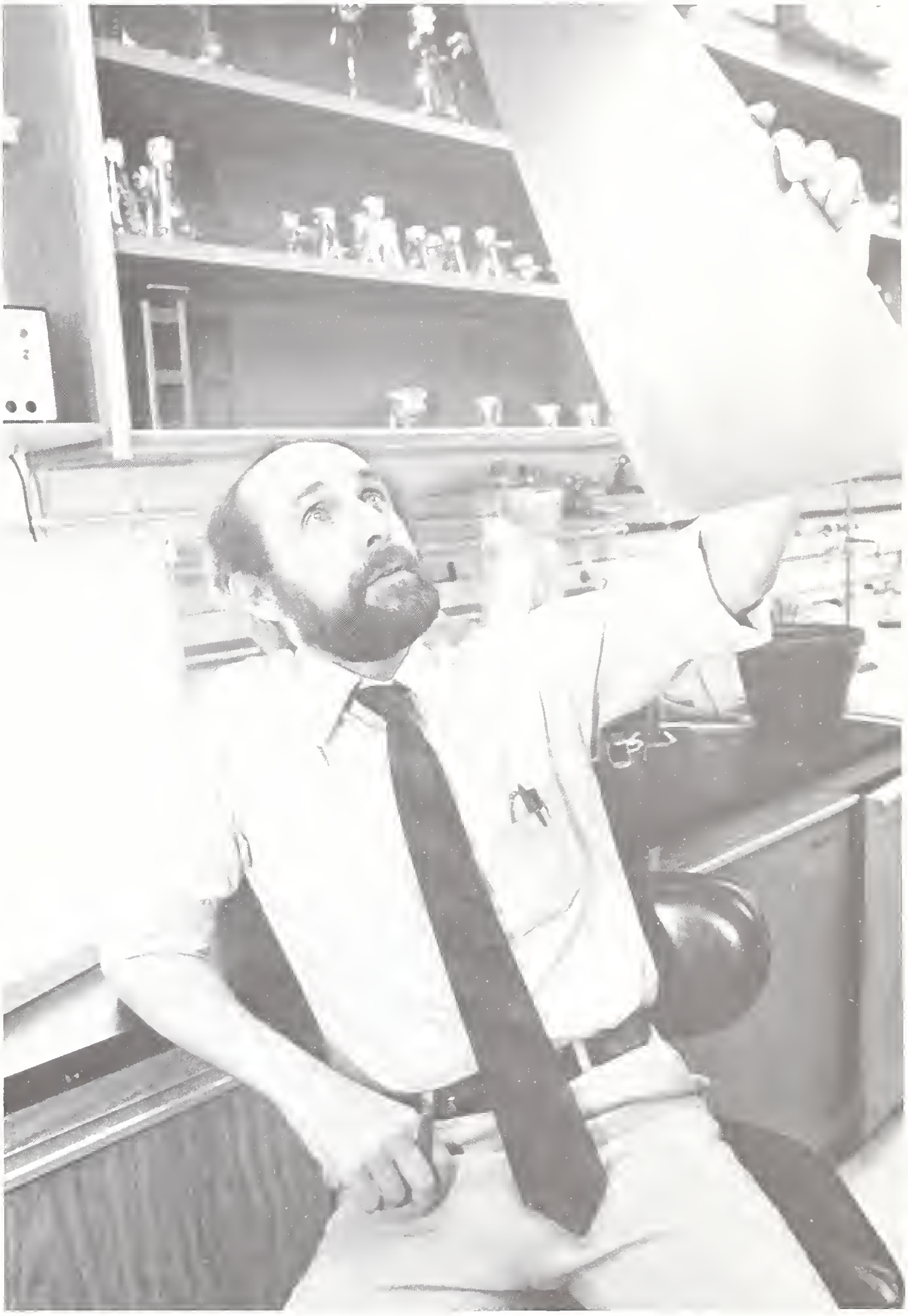
To do this, they radioactively tagged bits of DNA (called Alu sequences) that are commonly found in human DNA. With these they managed to successfully pull a fragment of the human gene out of the mouse DNA.

The next step was to isolate a whole functioning human gene. At this point, Dr. Friedmann's group borrowed a gene "library" prepared by Nobel Prize-winner Dr. Paul Berg and his associate, Dr. Hiroto Okayama, at Stanford. This "library" consisted of hundreds of thousands of unidentified pieces of human DNA. The human fragment that had been retrieved from the mouse DNA linked up with one of the "library" pieces. Dr. Friedmann's group was then able to clone the whole gene and place it in plasmids.

The most exciting parts of this story are yet to come. Dr. Friedmann is now studying HPRT gene organization and expression. He has nearly completed determining the nucleotide sequence of the gene. He has managed to insert his cloned HPRT genes into human lymphoblast cells that could not make HPRT. Cells thus transformed were able to make active enzyme.

Dr. Friedmann's success now makes it possible for researchers to isolate large amounts of this important gene. His work opens the door for studies of gene therapy that may, in the future, help victims of Lesch-Nyhan syndrome, gouty arthritis, and other genetic disorders.

The paper reporting the cloning of the HPRT gene was published in a recent issue of the *Proceedings of the National Academy of Sciences.* ■



Dr. Theodore Friedmann examines an autoradiogram which can enable him to determine when the HPRT gene is present in cultured cell colonies. **NIH PHOTO 1756**

Data Bank Provides Physicians with More Up-to-Date Information to Treat Stroke Patients

Elizabeth Pennisi

Computers in medicine: they keep track of patient records, aid in diagnosis, and monitor treatment. Now "information age" technology is helping the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) fight stroke, a crippling and sometimes fatal disorder afflicting over 400,000 Americans a year.

Findings from the NINCDS-funded Stroke Data Bank, just ending its three-year pilot phase, promise to advance stroke research and influence how physicians treat stroke patients.

The NINCDS Stroke Data Bank, the first national computerized storehouse of information about stroke patients, is providing a pool of stroke-related data that scientists can use to detect patterns in the factors causing this disease and in evaluating the effectiveness of therapy.

The pilot data were collected by Boston University, Duke University, University of South Alabama, and the University of Maryland.

NINCDS investigators are beginning to sift through the wealth of new information stored in the data bank. From early analyses of data from a group of patients in southern Alabama, scientists are confirming that certain strokes there are more common in blacks than whites. One early finding: in this group of patients, the incidence of strokes resulting from bleeding into the brain tissue itself is three times higher in blacks. These hemorrhages occur within the brain tissue when blood vessels, weakened by high blood pressure or other reasons, rupture.

More females, regardless of race, seem to suffer from strokes caused by subarachnoid hemorrhages. These strokes usually occur because a malformed blood vessel balloons out and pops, flooding the spaces between the brain and skull with blood.

According to data bank findings, strokes from either type of hemorrhage account for less than 15 percent of all strokes but, as has previously been suggested by other research, tend to be more deadly than other types of stroke.

More commonly, strokes result from infarcts—build-ups of dead cells, debris, and blood clots that clog blood vessels and cut off blood flow to parts of the brain. Sometimes the clot originates in the brain. In other cases a blood clot traveling through the body gets stuck in the brain's tiny vessels.

These traveling clots or emboli account for 17 percent of Stroke Data Bank cases, a higher percentage than investigators expected to find. Another 9 percent of strokes recorded in the Stroke Data Bank result from a subtype of infarcts which occur deep within the brain. Data Bank totals reveal that patients with these deep-seated infarcts have fewer complications and recover better than patients with other types of stroke.

Since January, 1980, the four university hospitals have compiled information on 1,158 stroke patients. For 18 months, physicians and nurse coordinators at each hospital recorded information on incoming stroke victims as well as patients who had strokes while hospitalized for other reasons. Investigators at the University of South Alabama also collected information about stroke victims from a geographically defined population in southern Alabama.

Prestroke histories, diagnoses, CT scans, laboratory test results, treatment, and information on how the patient progressed after the stroke were collected and stored in microcomputers, then transmitted electronically to a central computer at Stanford University Medical Center.

"Our major thrust," says Dr. Cynthia Gross, NINCDS statistician, "was to define stroke types and subtypes in conjunction with the most up-to-date lab techniques, including CT and angiography."

During the early 1970s, NINCDS, together with the World Health Organization, had established basic criteria for different types of stroke, but those criteria did not incorporate now available diagnostic techniques such as CT scan readings.

The participating Data Bank scientists agreed to split the two established stroke types—those caused by ruptured blood vessels, or hemorrhages, in the brain versus those resulting from clogged blood vessels—into subtypes. The more refined classification has helped highlight significant differences between subtypes. The scientists have found damage caused by some strokes seems to occur over a period of several hours, not instantaneously. Knowing this, investigators hope to develop treatment to curtail impairment from these evolving strokes.

"Relevant data from the pilot study have opened the door for research in intervention before the stroke occurs," says Selma C. Kunitz, project director for the NINCDS Stroke and Trauma Data Banks.

Stroke Data Bank investigators will concentrate on identifying the significance of prestroke symptoms. Data Bank physicians have noticed that certain stroke patients felt intermittent spells of dizziness or headaches prior to their stroke. Eventually, it may be possible to recongize these symptoms as warning signs and prevent the stroke from occurring with either surgery or drugs.

Four years ago many people involved in the care of stroke patients were skeptical about the value of a stroke data bank. Few people believed that physicians from different hospitals could standardize their procedures for diagnosis, follow-up, and recordkeeping enough to compile a reliable sampling of stroke victims.

"I think that there has been a lethargy and sense of hopelessness about stroke among physicians and investigators," says Kunitz. "The Data Bank has helped rekindle the interest of both neuroscientists and physicians."

Pilot project scientists have already begun to apply findings from the data bank studies to both their practices and their other research. The University of South Alabama started a program to diagnose stroke patients rapidly, using data bank results to help guide decisions about the treatment of incoming patients. At the University of Maryland, Dr. Thomas R. Price is studying the association between patients' psychological and social recovery, the size and location of damaged area of the brain, and the type of stroke.

The pilot Stroke Data Bank and another data bank for head trauma were initiated and organized by NINCDS Office of Biometry and Field Studies, and represent one of the first attempts to use "information age" technology to help fight neurological disorders. Over the next five years, in the main phase of the program, many more cases will be added to the stockpile of stroke data. And even the skeptics admit that, as research tools, data banks are potent disease-fighting "weapons" that are here to stay. ■

Pigeon Breeders' Disease Aids Researchers in Studying Lung Disorders

Research Resources Reporter

The triggers of allergic lung diseases are in the home and in white collar businesses, in grain silos and in freight cars, even in pigeon cotes.

Regardless of the source, the effects are always the same—impaired breathing, coughing, fever, and chills—the symptoms of a broad class of disorders called hypersensitivity pneumonitis.

Some people exposed to these allergens develop symptoms and others do not. The reason, according to researchers at the Medical College of Wisconsin (MCW) in Milwaukee, is that some people maintain a natural defense, cells that specifically suppress the effects of allergens.

When these cells—suppresor T-lymphocytes—fail to suppress the immune response, antibodies and sensitized cells attack and damage lung tissue, leading to the symptoms of hypersensitivity pneumonitis, says Dr. Jordan Fink, Chief of the Allergy and Immunology Section in the MCW Department of Medicine.

In studying pigeon breeders' disease, a lung allergy caused by inhaled pigeon matter, Dr. Fink and his colleagues discovered that a population of suppressor cells is active in the blood of healthy pigeon breeders, but not in the blood of patients with symptoms of the allergy.

Although pigeon breeders' disease affects only a small number of people, it may be a model for all forms of hypersensitivity pneumonitis. The symptoms are identical for all types of this disorder, Dr. Fink has found. A variety of bacteria, fungi, and protozoa can cause it.

These microorganisms can invade virtually every segment of society. They live in the heating and cooling systems of homes or businesses, contaminating the air breathed by residents or employees.

They may be present in foodstuffs such as grain and malt, exposing farmers and handlers on loading docks and in breweries to airborne allergens.

At present, researchers conclude that the activity of suppressor cells is the major determinant of hypersensitivity pneumonitis in pigeon breeders. This hypothesis is supported by tests indicating greater suppressor activity in the blood of asymptomatic subjects than in the blood of patients with the clinical signs of the disease.

Dr. Fink believes this heightened activity in asymptomatic subjects occurs throughout the body. If his hypothesis proves to be correct, the research may lead to an effective treatment or cure for hypersensitivity pneumonitis.

The study of pigeon breeders' disease is being funded primarily by the National Heart, Lung, and Blood Institute (NHLBI), the National Institute of Allergy and Infectious Diseases (NIAID), the Division of Research Resources (DRR), and the Veterans Administration.

Dr. Fink reports that preliminary evidence suggests that the activity of suppressor T-cells in the lungs of symptomatic pigeon breeders is not adequate to control the immune response to pigeon antigens.

NIH PHOTO 1757

The only effective treatment currently available for the disease is the use of corticosteroids, which can cause hypertension, decalcification of bone and numerous other adverse effects when used for a long time.

To make management even more difficult, neither avoidance nor treatment with corticosteroids is always enough. Symptoms, may persist in some patients, leading to chronic lung disease, which reduces the patient to a "pulmonary cripple," says Dr. Fink.

With pigeon breeders' disease as a model, the Milwaukee researchers hope to understand the defect that inactivates suppressor cells and makes people susceptible to allergens.

Patients with pigeon breeders' disease show clear-cut signs of the disease, thereby simplifying the diagnosis. Willing pigeon breeders participate in the research, providing financial support through their national organization and traveling across the nation to the DRR-supported General Clinical Research Center in Milwaukee.

In continuing their study of the disease, the first priority of Dr. Fink and his colleagues is to design a screening method for identifying susceptible individuals.

Their next priority is to design a therapy, one that will reactivate the protective mechanism that is dormant in patients with the disease.■

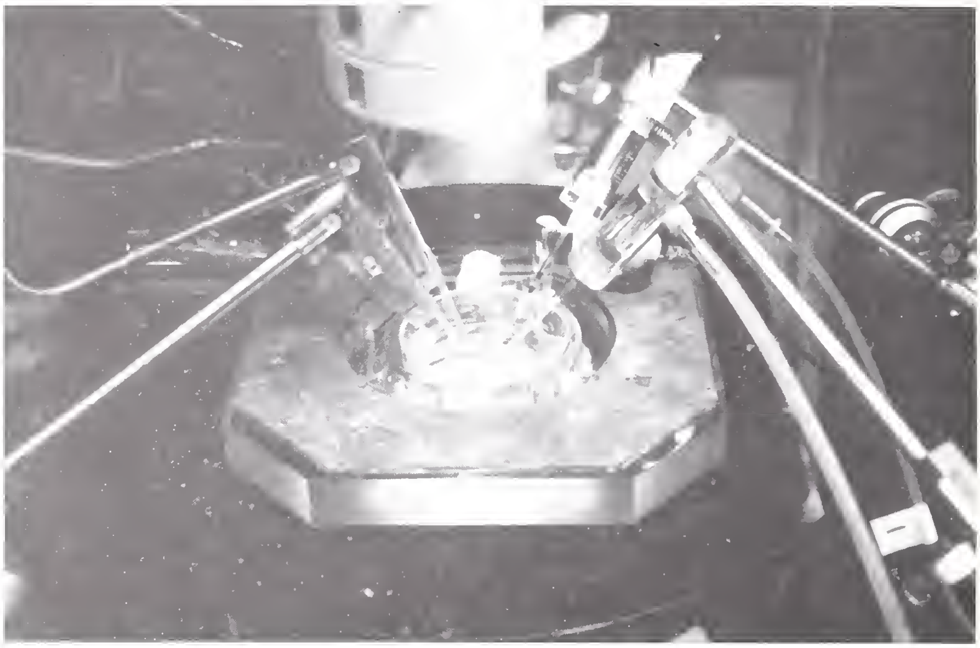
Milwaukee pigeon breeder, Carl C. Gill, has been very cooperative in recruiting fellow breeders to participate in allergy tests at the Division of Research Resources-supported General Clinical Research Center.

NIH PHOTO 1758

When Medicine Meets the Membrane—Understanding How Drugs Work

Elizabeth Pennisi

Academy of Sciences.



The microscope stage is where the action is in neurophysiology, the study of nerve cell properties. Needle-like microelectrodes inserted into isolated spinal cord cells measure minute changes in the cell's electrical properties.

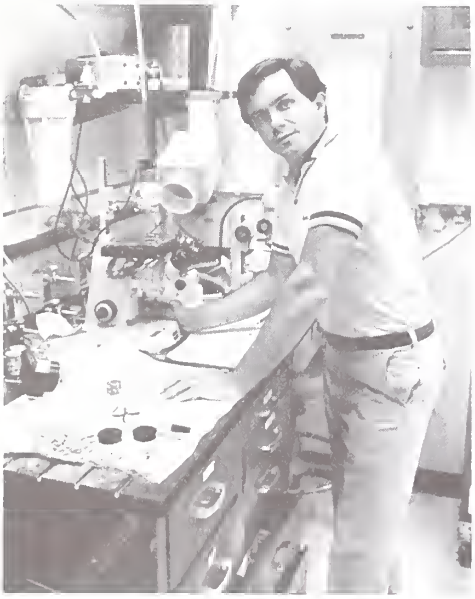
NIH PHOTO 1760

As any microscopic view of human tissue reveals, the body consists of millions of tiny cells. Each cell is surrounded by an almost invisible wall, the cell membrane. Magnified 1000 times or more, this wall can be seen as a fluid arrangement of larger particles imbedded in a thick jelly of smaller fatty molecules.

"The cell membrane is very important to the cell," says Dr. Jeffery Barker, Chief of the Laboratory of Neurophysiology at the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS). "It is the barrier that keeps ions and other substances in, or out."

The membrane is also very important to Dr. Barker, who has spent the last five years probing the secrets of one particular type of membrane—that of cells of the central nervous system, the nerve network's inner core consisting of the spinal cord and brain. From these investigations he and other NINCDS scientists are learning what role this membrane barrier plays in regulating the effects of drugs on the nervous system.

According to Dr. Barker, "excitability" is a key concept to understanding how nerve cells function. For a long time, however, scientists did not really know why only certain cells were excitable and therefore could transmit messages to other cells.



NIH PHOTO 1759

Dr. Jeffery L. Barker, Chief of the NINDCS Laboratory of Neurophysiology, and his colleagues spend many hours studying how drugs affect nerve cell membranes. He depends on a variety of sensitive techniques to help him understand how the membrane works

Investigators studying the nervous systems of simpler, soft-bodied animals like squid and snails found that the somewhat elusive property called excitability is created partly because of the membrane's ability to regulate the distribution of charged particles, or ions.

Ions bunch up inside or outside the cell and naturally want to redistribute themselves evenly across the membrane. Usually the membrane acts like a barrier, preventing the even distribution of ions. But when exposed to certain chemical or electrical stimuli, nerve cell membranes become "excited" and release ions from the cell or allow them to enter.

The resulting rush of ions creates an impulse, or action potential, that is transmitted along the length of the cell much like electricity traveling along high tension wires. These impulses initiate a chain of events for sending messages to the brain and the rest of the body.

Dr. Barker and his colleagues were intrigued by this excitability in simpler organisms, but did not know if the central nervous system cells of more complex animals—and man—worked the same way. Dr. Barker especially wanted to find out exactly how the chemical signals produced by drugs affect the membrane in man.

"If we don't understand that, then we will be left to depend on empirical judgment in using drugs," says Dr. Barker. "Drugs will be given because they work, not because we know how they work."

Unlike his peers who can examine excitability mechanisms in simpler but intact animals like the snail or squid, Dr. Barker must take apart the more complex and inaccessible nervous system of the mouse. His laboratory studies central nervous system cells isolated from mouse spinal cord and grown in culture.

But isolating central nervous system cells is tricky. First, a spinal cord is dissected from a 13 day-old mouse embryo and carefully minced. Then the small pieces are passed through a thin pipette which allows only small clumps of cells to pass at a time. This process helps pull the cells apart from one another. It is repeated until individual nerve cells separate out from the minced spinal cord tissue.

A few cells are placed on a small plastic plate coated with a substance suitable for nerve cell growth. Within a few weeks, the cells that have survived the mincing and sifting develop into a single layer or monolayer of living spinal cord tissue.

Although the monolayers of cells lack the organization of an "intact" nervous system, these cultures react to drugs and other substances just as nerve cells would in the body. But because the cells are in single layers and not buried deep within the body, Dr. Barker and his colleagues can study them in greater detail.

"This monolayer allows easy accessibility to many neurobiological techniques," says Dr. Barker. "It's like being able to examine moon dust and moon rocks right on the lunar surface, rather than trying to study them through a telescope from here on earth."

Tiny electrodes the size of fine needles can be inserted directly into the isolated cells. These sensitive electrodes monitor small changes in the cell's electrical properties that occur when the membrane becomes excited and ions rush in and out of the cell. Using this and other techniques, Dr. Barker has gained insight into living nerve cell membrane properties.

Neurophysiologists at NINCDS have learned that, as in simpler organisms, membranes of cultured central nervous system cells contain a maze of channels that regulate which ions pass in or out of cells. When cells are stimulated, channels open up just long enough to let ions through the membrane. Otherwise, mechanisms that work like gates prevent those charged particles from leaking through membrane channels.

This "two-state" channel system—in which the membrane alternately lets ions through, then holds those same ions in or out of the cell—is crucial to maintaining the nerve cell's ability to receive and transmit signals.

Dr. Barker and his coworkers are beginning to understand just how certain clinically important drugs, including anticonvulsants, work at the membrane level. These drugs seem to affect the activity of one of the nervous system's most important chemical messengers, gamma-aminobutyric acid (GABA). GABA calms excitable nerve cells. It is one of several messenger chemicals that inhibit the production of impulses generated by nerve cells.

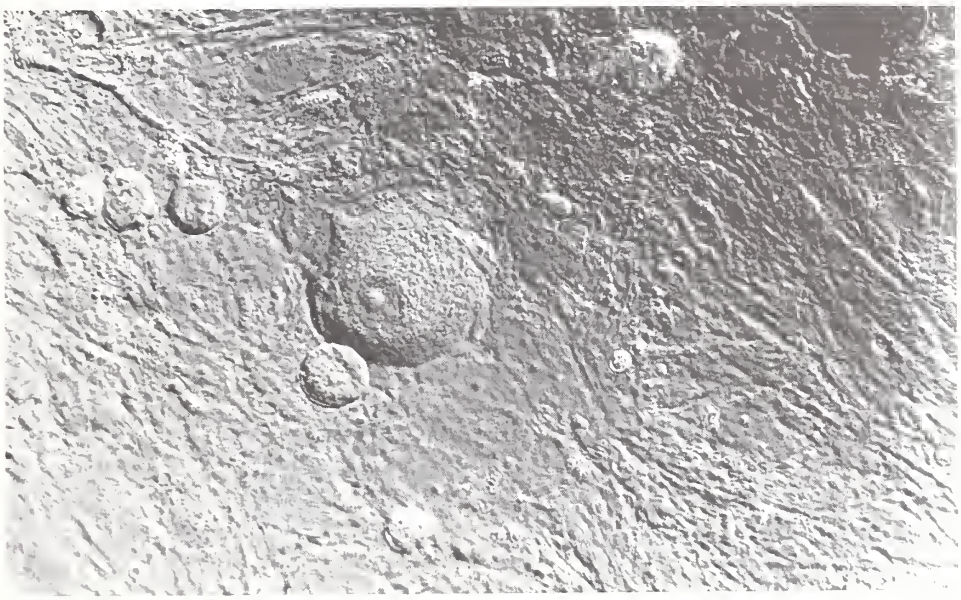
Acting as a kind of gatekeeper, GABA helps dampen the excitability of nerve cells by selectively opening channels that let negatively charged chloride ions enter the cell. By letting more chloride ions in, GABA helps make the cell less likely to give off unwanted impulses that might lead to seizures, or feelings of anxiety.

Valium(R) (a trade name for a drug called diazepam), barbiturates, and other substances enhance GABA's ability to open chloride ion channels and thus keep cells from being too excitable.

Scientists at th NINCDS Laboratory of Neurophysiology have discovered that not all drugs enhance GABA the same way. Dr. Barker and his colleagues have found that drugs like Valium(R) work by somehow telling GABA to open the channels more frequently. Barbiturates seems to work by keeping the channels open longer. In both cases GABA lets extra ions enter.

At NINCDS, investigators are examining exactly how these and other drugs interact with GABA's gatekeeping functions. Other NINCDS laboratories continue their probe into the specific nature of the chemical and electrical imbalance that the membrane seems to control.

As Dr. Barker points out, understanding these membrane processes is a step toward understanding how the brain functions and unraveling the true nature of neurological diseases—and a step closer to preventing and curing them. ■



This "lunar" surface is actually the surface of a spinal cord cell highlighted by a special technique causing light to hit the cell at a very low angle. The "crater" is really the cell nucleus.

NIH PHOTO 1761

The isolated spinal cord cell, when grown in culture, is easier to test than nerve cells of intact animals. These cells, derived from the spinal cord of mouse embryo, help neurophysiologists better understand nerve cell membrane properties.

NIH PHOTO 1762

NICHD Celebrates 20 years of Achievement

Maureen Gardner

A Federal agency whose research has helped thousands of children reach their first birthday in good health is celebrating its 20th anniversary this year. Without the research of the National Institute of Child Health and Human Development (NICHD), many healthy children alive today would have died or suffered lifelong disabilities.

The impetus to create the NICHD came in 1961, when a task force report to President Kennedy charged that research into the physical, intellectual and emotional growth of the child was "severly handicapped" by the absence of a central coordinating point. It called for a new Institute at the National Insititutes of Health to launch a "concentrated attack" against disorders of human development.

That request ultimately changed NIH history. Solutions to problems of physical and mental development had to be based on a better understanding of *normal* human biology and behavior. At the time, by law, each Institute was designed to wage battles against a specific disease. The Congressional mandate had no provision for an Institute with a major interest in normal human reproductive, growth, and developmental processes.

A new law had to be written. On Oct. 17, 1962, a totally different pattern of research effort was set in motion through President Kennedy's authorization of the National Institute of Child Health and Human Development.

NICHD's creation symbolized an emerging concept: while we continue to search for solutions to specific disease problems, we must also probe more deeply into all the life processes—behavioral and biomedical, normal and abnormal—as a basis for maintaining health as well as for the prevention and conquest of disease.

The Institute's search for understanding has followed the continuum of life itself. Studies have explored events before conception, including basic reproductive biology and attitudes toward contraception and parenthood. Research has moved through the transformation of a fertilized egg into an infant; the physical and psychological growth of an infant into a child, adolescent and adult; and the complexities involved in childbearing and childrearing.

Thousands of research studies have been performed with NICHD funds—more than 1,300 in 1981 alone. The goals of these studies have been as difficult as they are important:

- Programs have aimed to assure the birth of *healthy babies* through investigations on nutrition, normal and abnormal fetal development, and problems of pregnancy.

NIH PHOTO 1763

- Many projects have sought to assure the birth of *wanted* babies through studies on human fertility and infertility, better methods of family planning, and adolescent childbearing.
- Others have aimed to provide *all children* the opportunity for productive and healthful adulthood through efforts to understand the causes of mental retardation, birth defects, sudden infant death syndrome, and learning disorders, as well as normal development and growth.

NICHD's unique approach has yielded great returns in the past two decades. Working toward the goal of healthy babies, Institute-funded scientists made improvements in the treatment of premature infants that contributed to a 22 percent drop in infant mortality in the U.S. between 1976 and 1980. That decline represents the prevention of some 10,000 infant deaths each year.

The maternal death rate from pregnancy and childbirth has also fallen dramatically—an 80 percent drop between 1962 and 1980—owing in large part to NICHD-supported research advances that allowed women to control their fertility and improved the management of life-threatening conditions in pregnant women.

Efforts toward the goal of assuring the birth of wanted babies have resulted in treatments for male and female infertility, as well as the development of improved family planning methods. They have also led to much safer use of currently available contraceptives.

Scientists striving toward the goal of assuring a productive adulthood for all children have made great steps in preventing certain types of mental retardation. They have developed screening tests and treatments for some inherited metabolic disorders that cause mental retardation.

When the Institute was formed, its first order of business was to correct a problem emphasized repeatedly by obstetricians, pediatricians and other specialists at the Congressional hearings in 1961: the serious shortage of research personnel in the field. In the last two decades, NICHD responded to that need by supporting the training of more than 4,000 research scientists in the areas of pregnancy, child health and human development.

A few NICHD research highlights follow. They show the diversity of NICHD research, which traces the path of human development and spans the normal and abnormal, the biomedical and behavioral, the basic and clinical.

Understanding the Basics

A better understanding of the two basic components of fertility—egg and sperm—has been achieved through many NICHD-supported projects. Grantees identified two hormones that influence sperm production in men and the monthly release of an egg, or ovulation, in women. Intramural scientists isolated the receptor molecules on the ovaries and testes that allow them to respond to hormonal signals that promote ovulation and sperm production.

A substance that inhibits the maturation of egg cells was discovered by one group of grantees, and another group identified enzymes that facilitate the penetration of a sperm cell into an egg. These and other findings have enhanced our understanding of basic reproductive processes, and form a basis for future medical applications.

Investigating Prematurity

Research aimed at reducing the number of infants born too early or too small, like the 3½-pound baby girl photographed on the cover, has been a priority of NICHD. In order to prevent premature birth, the mechanisms involved in the initiation of labor must be understood.

Recently, grantees found that the same enzyme released by the amnionic membrane to start labor is also produced by certain bacteria that can infect the female reproductive tract. Investigators are now testing the possibility that the bacterial enzyme may initiate premature labor. If confirmed, this could be a major cause of premature labor, and one that could be readily prevented.

Testing for Lung Maturity

Each year, about 40,000 newborns develop respiratory distress syndrome (RDS). These babies cannot breathe on their own because their lungs are immature. A prenatal test for fetal lung maturity developed with Institute support has become invaluable in managing premature labor and cesarean delivery, two situations where the risk of RDS can be high.

If the test shows the fetal lungs to be immature, it is often possible to prolong pregnancy until the lungs mature, or give the mother hormones to hasten fetal lung development. When RDS occurs despite all efforts, special air pressure treatments which keep the lungs inflated, have markedly improved infant survival.

Breast Milk Is Best

NICHD-funded research has virtually confirmed that breast milk is the ideal food for normal, full-term infants, for at least the first six months of life. Among the advantages of breast milk are its protein quality, digestibility, low inorganic salt content, and calcium, phosphorus, and vitamin C content.

NIH PHOTO 1764

In addition, breast milk seldom evokes an allergic response, and it conveys immunological benefits. Based on this research, medical groups are encouraging breastfeeding and the percentage of women who breastfeed is increasing. (Photo: Baylor College of Medicine.)

Father-Child Interplay Studied

In recent years, interaction between fathers and their children has been studied in many NICHD projects. Investigators have found that toddlers seek out their fathers, are disturbed by separation from them, and at times prefer their fathers to their mothers.

NIH PHOTO 1765

New studies by intramural researchers show that infants as young as six months old are more persistent in problem-solving tasks if their fathers play with them. The studies found that even though fathers spend much less time with their infants than mothers do, they have a greater effect on problem-solving behavior.

Preventing Mental Retardation

Thousands of cases of mental retardation have been averted through advances made by Institute-supported scientists, who continue to search for other preventive measures.

Research by some of these investigators led to development of a screening test for newborns with thyroid glands that are either absent or not working properly. Without treatment, this condition—called congenital hypothyroidism—leads to mental retardation. The test allows early detection and treatment, greatly improving the prognosis for normal mental and physical development.

Understanding Down Syndrome

Children with Down syndrome tend to remain isolated from nonretarded children unless adults intervene. NICHD-supported researchers have found that social exchanges between retarded and nonretarded preschoolers can be greatly enhanced by training the children to initiate contact and respond to one another

This type of research has contributed to improved home care and public education, and a fuller role in community life for persons with Down syndrome. Institute-funded biomedical studies have led to new approaches in genetic counseling and in research design.

In addition, NICHD researchers recently identified in mice a chromosome anomaly that can be used as a model for the Down syndrome genetic defect. The mouse model will allow basic experiments which are essential, yet impossible to perform in humans.

Research on Reading

Reading, far more than speech, places a direct demand on a person's knowledge of language, Institute-funded studies show. From its beginning, NICHD has supported research on reading disabilities. This research has led to the development of tests that can be used to diagnose reading problems in children and to prescibe corrective measures.

Birth Control Evaluated

Assessing the safety of birth control methods used by millions of men and women is a major part of NICHD's program in population research. Studies on oral contraceptives have demonstrated the dose-risk relationship, and helped define high-risk populations, such as smokers and women over 35. As a result, pill formulas have improved, prescribing practices have changed, and consumers are able to make more informed decisions.

Institute-funded studies recently confirmed some of the pill's noncontraceptive benefits, such as protection from pelvic inflammatory disease and cancer of the ovary and uterine lining. Other major studies have focused on the side effects of the intrauterine device, and several large projects evaluating the safety of vasectomy are in progress.

The increasing popularity of barrier methods of birth control has prompted Institute-funded development and evaluation of new spermicides, diaphragms and cervical caps, as well as large-scale trials of existing barrier devices.

These examples make up a tiny fraction of the many valuable research findings achieved through NICHD-supported studies. For more information, write to the NICHD Office of Research Reporting, NIH, Building 31, Room 2A32, Bethesda, Maryland 20205. ■

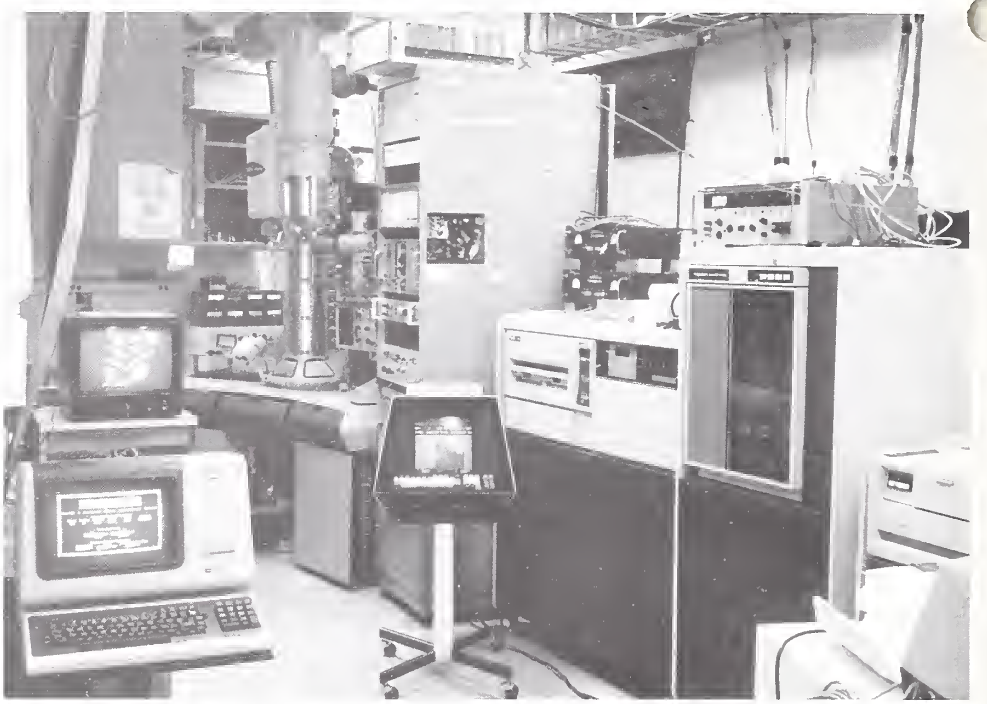
Sophisticated Electron Microscope Developed by DRS and DCRT

Joyce McCarthy

A sophisticated new instrument to analyze and display a specimen's elemental makeup as well as its morphological microstructure has been developed by NIH scientists. This new electron microscope imaging system, composed of an analytical electron microscope (AEM) and a digital computer, correlates a specimen's microstructure with its microchemistry on a scale of a hundred atom diameters. It is available for collaborative research use by all NIH scientists.

The NIH system is the only one of its kind in the United States. Only a few laboratories in the world can perform the specific type of chemical microanalysis done here; moreover, the spectrum of capabilities of the new NIH system is unique.

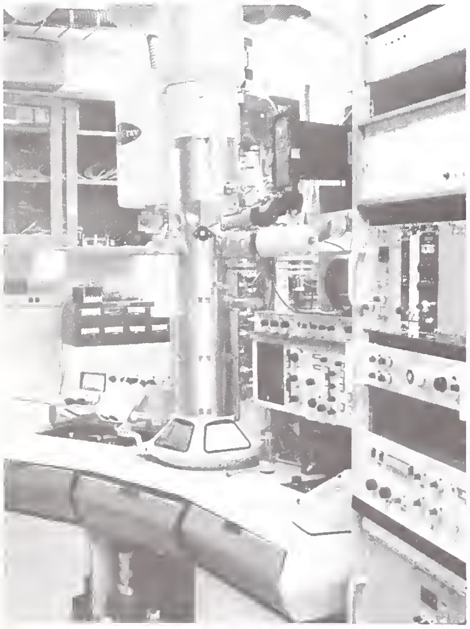
The imaging system is the result of four years of intensive work by a project team formed from the Division of Computer Research and Technology and the Division of Research Services. Physicists, engineers, and computer scientists from DRS' Biomedical Engineering and Instrumentation Branch and DCRT's Computer Systems Laboratory collaborated to develop this complex, sophisticated technology.



The Analytical Electron Microscope (rear) and online computer with the color display (left foreground) are housed in Building 13.

NIH PHOTO 1767

Incorporating an electron microscope, the new analytical instrumentation chemically analyzes a specimen. Digital computers collect and process data and produce an image on a color display. Colors in the image represent the distribution of different elements in the specimen.



The Analytical Electron Microscope can do everything a conventional electron microscope can do, but now, the team has designed the microscope to chemically analyze a specimen and show the results on a background-corrected color display.

NIH PHOTO 1766

Computer system hardware and software, modifications to the electron microscope, and methodologies for actual specimen analysis all were developed by members of the team. The instrument is a good example of interaction among people of different skills and training producing a novel scientific research tool more powerful than one produced by scientists of a single discipline.

The analytical electron microscope performs all the jobs conventional electron microscopy can do—conventional transmission EM, scanning transmission EM, energy dispersive X-ray EM, and electron energy-loss spectroscopy—but the AEM also produces images of the distribution of the chemical elements.

The electron beam is scanned under computer control over a region of the specimen while selected signals (spectra) acquired from many thousands of points in the specimen are stored directly in the computer rather than on a photographic media, like a conventional electron microscope.

These stored signals are then processed to produce images on a color display to permit correlation of elemental distributions and morphology. Under computer control, a number of signals can be recorded simultaneously to obtain images of the distributions of all elements in the periodic table except hydrogen and helium.

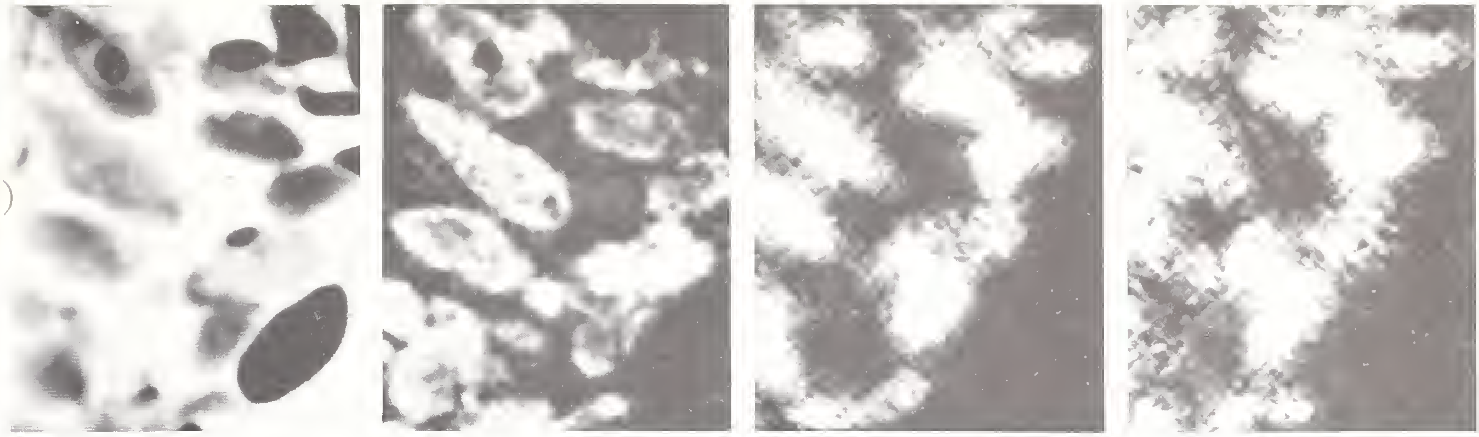
The signals used to produce the chemical images are of two kinds: the electrons which have lost a characteristic amount of energy in interacting with the atoms of a specimen; and the characteristic X-rays produced during the interactions.

Both of these are spectroscopic in nature. In other words, the measurements are made on the spread of electron energies similar to the spread of wavelengths produced when light passes through a glass prism to form different colors.

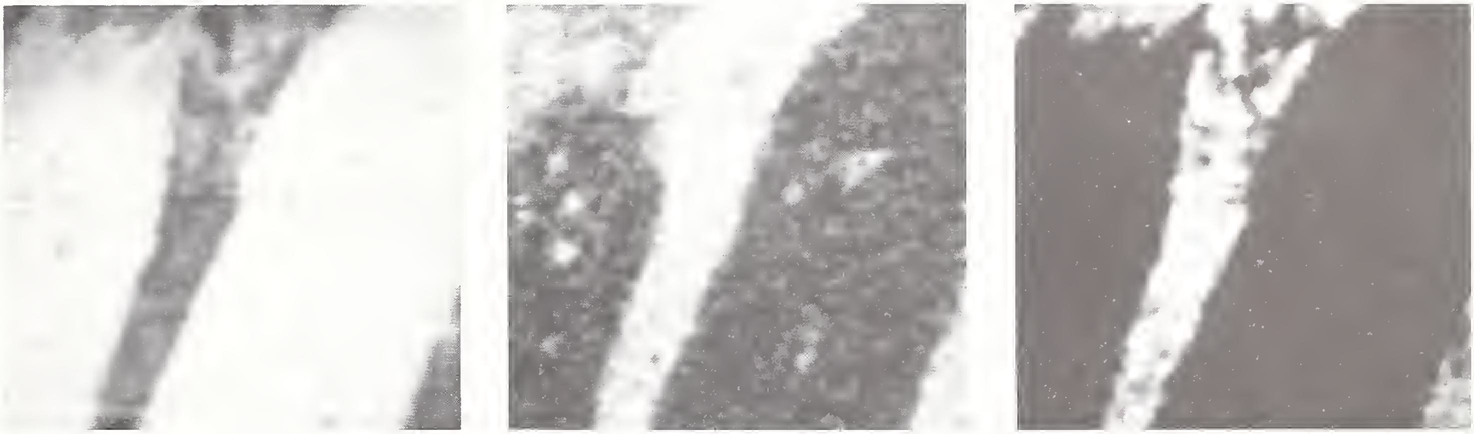
The signals are produced when electrons traveling nearly one-half the speed of light pass through samples placed inside the electron microscope, creating ionization in the thin section of specially prepared tissue.

The incident beam of electrons in the microscope interacts with the specimen atoms, and some of their tightly bound (core) electrons are ejected.

When this occurs, the beam electron loses an amount of energy (characteristic of the atomic species), producing electron energy-loss spectra. Then, when the now-ionized atom relaxes, an X-ray can be emitted which is also characteristic of the atom, producing X-ray spectra.



These elemental images are of a primitive blood cell from a horseshoe crab containing secretory granules. The photo on the left is an Electron Energy Loss Spectroscopy (EELS) image of carbon, the second photo from the left is an EELS image of nitrogen, the second photo from the right is an X-ray image of sulphur and the photo on the right is an X-ray image of calcium. **NIH PHOTO 1768**



The three elemental images are of ameloblasts, or enamel-forming cells taken from a rat's incisor. The photo on the left is an Electron Energy Loss Spectroscopy (EELS) image of carbon, the center photo is an EELS image of nitrogen, and the photo on the right is an EELS image of calcium. **NIH PHOTO 1769**

Both of these signals can be utilized individually or together to perform a chemical analysis of one isolated microvolume. Alternatively, the electron beam can be scanned across a region of the specimen and an image of the chemical information can be obtained.

The digital computer is crucial to the analytical imaging system. Its speed and storage capacity make possible the high speed acquisition of the huge quantity of data generated as the electron beam is electronically scanned over the specimen, and the complex mathematical manipulation necessary to produce elemental images from the raw data.

Major effort also has been put into designing software that is both easy to use and flexible enough to deal with a broad range of experiments.

The analytical imaging system can be applied to a wide range of problems, for example, elemental distributions in bone samples, tumor cells, or brain tissue from patients suffering from Alzheimer's disease.

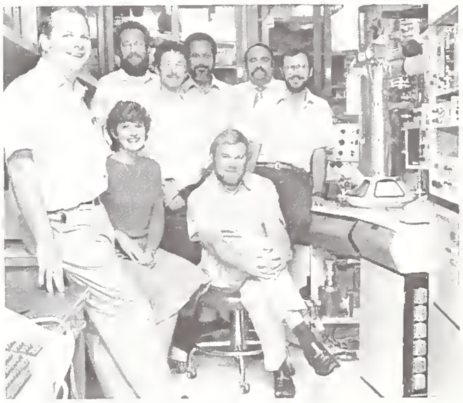
Also available in this laboratory is an electron beam microprobe, a high-quality scanning electron microscope on which elemental X-ray microanalysis can be performed on bulk as well as thin specimens.

In the future, the group will extend computer control to the microprobe. This will permit elemental imaging of bulk specimens and stereology, or measurements of shapes and features.

Any of the analytical facilities are available to researchers at NIH wishing to collaborate on an appropriate project, either basic or applied.

Scientists currently using the system are investigating the localization of calcium and nitrogen in a number of cell types, as well as the distribution of fluorine-labeled neurotransmitters.

For more information on this new technology, call the Electron Beam Imaging and Microspectroscopy group, 496-2599. ■



Members of DCRT and DRS seated left to right are: Carol Swyt and Chuck Fiori, and standing left to right Carter Gibson, Luther Borden, Richard Leapman, Jim Ellis, Jim Delpriori, and Keith Gorlen. **NIH PHOTO 1770**

Rimantadine and Amantadine Studies in Preventing Influenza A

Pat Randall

Two related drugs, amantadine and rimantadine, effectively protect against influenza A infection, but rimantadine produces significantly fewer side effects, according to a recent study.

These results suggest that rimantadine is the drug of choice for prevention from influenza A. Until now, the effectiveness of rimantadine had not been documented in a strictly controlled, large clinical trial.

Amantadine has been approved for prevention and treatment of influenza A since 1976, but has not been widely used. Rimantadine is approved only for experimental use although it is used abroad, particularly in the Soviet Union.

In this study with young, healthy volunteers, both drugs were effective, but amantadine produced more central nervous system (CNS) side effects, mainly insomnia, jitteriness, and difficulty in concentrating. The side effects disappeared however, within 48 hours after medication was discontinued.

Scientists at the University of Vermont College of Medicine began their study during the early stages of a 1981 influenza outbreak in Burlington, Vt. Four hundred and fifty volunteers, aged 18 to 45 years were enrolled in a controlled double-blind trial.

Study participants were given tablets twice a day for six weeks. One-third received amantadine; one-third received rimantadine; and one-third received a placebo, an inactive substance.

Both rimantadine and amantadine were effective in preventing influenza-like illness, defined as a cough and/or fever, and at least two of the following symptoms—sore throat, headache, or muscle aches.

When compared with placebo, rimantadine reduced the rate of illness 65 percent and amantadine reduced the rate 78.2 percent.

The effects were even more striking when the investigators analyzed the reduced rates of laboratory-confirmed influenza A illness. Rimantadine decreased this rate by 85.4 percent, and amantadine lowered the rate by 91.2 percent, when compared with placebo.

These findings also indicate that some influenza-like illnesses were not caused by influenza A viruses, but by other organisms not receptive to these medications.

CNS side effects developed in 13.1 percent of the volunteers receiving amantadine, 6.1 percent of rimantadine recipients, and 4.1 percent of placebo recipients. The frequency of other side effects, such as gastrointestinal problems, was the same for all groups.

CNS side effects were responsible for a higher rate of withdrawal by amantadine recipients from the study. (Of amantadine recipients, 22.1 percent withdrew, compared with 10.8 percent of placebo recipients, and 9.5 percent of rimantadine recipients.)

Laboratory tests on all volunteers revealed that several had evidence of influenza A infection even though they had had no symptoms of illness. Both drugs seemed less effective in preventing *infection* with influenza A virus than in preventing infection-associated illness.

Compared with placebo, rimantadine reduced the rate of infection 65.7 percent. Amantadine reduced this rate 74.4 percent. This phenomenon may be desirable, however, if these subclinical (inapparent) infections provide immunity to subsequent infections with the same or related influenza A viruses.

The scientists recommend that additional studies be done to determine whether results, including the low rate of side effects, would be similar in elderly or other high risk persons.

This study was supported by contract from the National Institute of Allergy and Infectious Diseases. The investigators reported their findings in the Sept. 2, 1982, issue of the *New England Journal of Medicine*. ■

Autologous Bone Marrow Transplantation May Permit More Extensive Cancer Therapy

Ann Menting

National Cancer Institute (NCI) scientists are exploring ways to sidestep the toxic effects that anticancer drugs have on bone marrow. Through a procedure called autologous or "self" bone marrow transplantation, a sample of a patient's bone marrow cells is removed before irreversible or severe damage occurs. The bone marrow is then frozen and later can be returned to the patient for therapy, if needed.

Doses of chemotherapy have been limited because of their destructive effect on bone marrow cells, particularly on the precursors of white blood cells and platelets. This transplantation technique, however, may allow patients to receive larger and more effective amounts of anticancer drugs.

The autologous bone marrow procedure begins with withdrawal of marrow from the patient's pelvic bone, usually while the patient is under general anesthesia. The marrow is then prepared for freezing by the addition of demethysulfoxide (DMSO). The DMSO keeps the water in the marrow cells from forming crystals as it freezes—crystals that could damage the cell membrane. The marrow cells are then stored in liquid nitrogen at -196°C which keeps about 80 percent of the cells viable for up to eight years. When the patient is ready, the cells are thawed quickly in warm saline and are infused intravenously. They then travel to the bones and grow into functioning marrow.

Research with autologous bone marrow transplantation indicates it may be useful for patients receiving extensive radiation therapy. In 1957, Dr. Kurnick o Long Beach Veterans Administration Hospital in California transplanted the first human autologous bone marrow in patients receiving radiotherapy. Although the procedure was not widely adopted at that time, its therapeutic value was recognized. Researchers at a number of institutions began studying and refining the technique and found that bone marrow transplantation appears most helpful in diseases in which the marrow cells are either defective or nonfunctional, such as immune deficiency diseases, aplastic anemia and leukemia.

During the 1960s, scientists studying marrow cell diseases looked at bone marrow transplants from donors whose marrow closely matched the marrow of the patient. In these types of transplantations, the donor is an immunologically matched twin or sibling. Despite careful effots to transplant only closely matched cells, many patients developed graft-versus-host (GVH) disease, a potentially fatal disease in which the donated bone marrow "attacks" the patient's tissues. Improved matching techniques have lessened the incidence of GVH, but it is still a major problem. However, in autologous transplants, the GVH problem is circumvented because the patient's own marrow cells are used.

Intense study of autologous bone marrow transplantation began at NCI in 1974, and more than 70 patients with various forms of cancer have undergone the procedure here. The technique has been used in adults with testicular carcinomas, undifferentiated lymphomas, and small cell lung carcinomas, and in children with Ewing's sarcoma, lymphomas and rhabdomyosarcoma. In almost all of these patients, the bone marrow transplants were successful, producing healthy bone marrow within about three weeks. Clinical studies of autologous bone marrow transplantation also are under way at Johns Hopkins University in Baltimore; the University of Washington in Seattle; M.D. Anderson Hospital and Tumor Institute in Houston; the University of Colorado Health Sciences Center in Denver; and Duke University in Durham, among others.

NCI scientists now plan to expand this procedure to patients with other types of cancer. Future studies will also ascertain if the high-dose chemotherapy permitted by the procedure is really more effective. Other studies are investigating ways to clear the marrow of tumor cells before infusion, a procedure necessary for patients with cancers like leukemia. A preliminary report from Boston's Sidney Farber Cancer Institute indicates that monoclonal antibodies that can recongize tumor cells may be useful in solving this problem. ■

Researchers Discover High Rheumatoid Factor in Elderly Citizens

Immunological cells taken from the elderly produce more of the autoantibody known as rheumatoid factor. Scientists from the University of New Mexico School of Medicine in Albuquerque, in research supported by the National Institute on Aging and the Division of Research Resources, found significantly higher levels of rheumatoid factor in blood samples taken from 31 healthy donors over the age of 70 than in samples from 27 young donors.

Lymphocytes from the elderly donors also produced more rheumatoid factor in culture. During experiments designed to find out which lymphocytes were involved, the investigators discovered abnormalities in both T and B lymphocytes. Drs. Martin A. Rodriquez, Jan L. Ceuppens, and James S. Goodwin published their rheumatoid factor study in the June 1982 issue of *The Journal of Immunology*.

A big problem in old age is that the immune system often manufactures larger-than-normal amounts of autoantibodies, substances that attack the body's own cells rather than foreign invaders. Many scientists believe that autoantibodies are responsible for certain age-related diseases.

Rheumatoid factor is one of the autoantibodies frequently found in blood sera of healthy old people, and the New Mexico team has been trying to define the immunological defect responsible for the increased production of these cells.

The scientists tested blood samples and lymphocytes from 27 healthy young people, aged 21 to 35, and 31 men and women over 70. The elderly donors were part of a long-term study of health of old people. All were in excellent health.

Lymphocytes from each donor were incubated with pokeweed mitogen, a substance that stimulates cellular activity. Stimulated immune cells from old donors, the investigators found produced significantly more rheumatoid factor than young lymphocytes. The increased autoantibody production by old lymphocytes paralleled the higher levels of rheumatoid factor found in blood samples from old donors. In further tests, the investigators separated T from B lymphocytes and assessed the effect of each on autoantibody production.

The tests revealed that old T cells provide more "help" than young cells to produce antibodies by B lymphocytes from either young or old individuals. Old B lymphocytes, however, proved to be less capable than young B cells of producing rheumatoid factor, given the same degree of stimulation. The scientists think the primary defect in the aging immune system may lie in the declining function of B cells. The T cells become more active as they try to compensate for the loss of B lymphocytes function.

The New Mexico investigators commented that their results support the suggestion of other investigators that a basic functional B cell defect during aging may be responsible for the secondary T cell dysfunction.■

NIH PHOTO 1771

Aluminum: Does it Contribute to Alzheimer's Disease?

Marian Emr

A major new finding reported in the Sept. 10 issue of *Science* has linked the results of two separate avenues of research on Alzheimer's disease—research that suggests that aluminum may contribute to the disease's development, and research that probes the excess incidence of dementia in three separate populations. Alzheimer's disease is the most common form of "senility" among the elderly.

In a decade marked by major advances in the fields of neuroscience and aging research, scientists have employed a variety of methods to investigate the possible cause(s) of Alzheimer's disease.

Their studies have looked at the roles of heredity, trace metals, deleterious changes in brain chemistry, and slow-acting viruses, as well as the effect of changes in the aging immune system.

This latest finding focuses on the hallmark of Alzheimer's disease, the presence of tangled fibers in the brain cells of victims.

With grant support from the National Institute on Aging (NIA), Dr. Daniel P. Perl of the University of Vermont has extended his earlier findings of accumulations of aluminum within the affected nerve cells of subjects showing the classic neurofibrillary tangles of Alzheimer's dementia.

Dr. Perl and Drs. D. Carleton Gajdusek, Ralph M. Garruto, Richard T. Yanagihara, and Clarence J. Gibbs, Jr., of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) report the new finding of high accumulations of aluminum, iron and calcium in the brains of Chamorro natives of Guam who have died of amyotrophic lateral sclerosis (ALS) or parkinsonism-dementia.

This population is adversely affected by these two chronic disorders, both of which were previously suspected to be transmitted by a slow-acting virus.

For several years, Dr. Gajdusek and his NINCDS colleagues have been studying the possible implications of Guam's environmental deficiency in calcium and magnesium and excess of aluminum and other metals.

Researchers on aging have kept a watchful eye on studies in Guam because of the similarities between the parkinsonism-dementia syndrome and the presenile form of Alzheimer's dementia. In both disorders, there is an excessive accumulation of neurofibrillary tangles in the brains of victims that is associated with severe dementia and death.

These latest findings reconfirm Dr. Perl's earlier work in support of studies linking high concentrations of aluminum in the brain to the development of neurofibrillary tangles, as well as work of Japanese investigators in both Guam and Japan.

They also lend support to a developing hypothesis that environmental factors may be an underlying cause of the ALS and dementia so prevalent in Guam.

NINCDS scientists are exploring the possibility that secondary hyperparathyroidism caused by lack of calcium and magnesium in the environment provokes an accumulation of metal ions within brain tissue.

Samples of garden soil and drinking water in Guam, as well as in parts of Japan and New Guinea that are similarly plagued by high incidences of ALS and dementia, indicate low levels of calcium and magnesium and unusually high levels of aluminum.

Scientists have never been able to prove that aluminum alone predisposes a person to, or causes, Alzheimer's disease.

Future studies will determine whether accumulations of metals in the brain are a primary cause of Alzheimer's disease, or if other factors or circumstances might combine with environmental factors to trigger the onset of chronic, but ultimately fatal, diseases of the nervous system.■

NIMH Studies Mood Disorders and Menstrual Cycle

Judy Folkenberg

Why does the depression of some manic-depressive patients "switch off" when their cycle of sleep and wakefulness is altered? Why is the depression of others ameliorated by prolonged exposure to light—a therapy which mimics the light-lengthened days of spring?

A National Institute of Mental Health (NIMH) intramural scientist is attempting to address these questions by focusing on another cyclic phenomenon: mood disorders associated with menstruation.

"Within a single day, the rhythms of biology can play a critical role either in the expression or development of affective disorders," says Dr. David Rubinow of NIMH's Biological Psychiatry Branch.

In the course of his clinical work, Rubinow reports, he has seen several women who experienced postpartum depression and later developed a menstrually-related mood disorder, followed by a major manic-depressive illness.

"I'm not suggesting that a menstrually-related mood disorder leads to a manic-depressive illness," he explains, "but I am suggesting that mood disorders associated with menstruation might serve as a sensitizing stimulus, and this may have an impact on the expression of an affective disorder in *some* women."

"The research I am doing is just another piece of the puzzle in this fascinating area where a biological switch is thrown and another mood state is ushered in," comments Rubinow.

"Biological switches are one of the most powerful tools we have to study, and women with menstrually-related mood disorders are wonderful models to see how the switching mechanism influences moods."

To determine "what takes place electrophysiologically, biochemically, neuroendocrinologically, and phenomenologically," Rubinow has structured his research project to avoid major methodological problems of past studies on "premenstrual syndrome" (a term he finds inaccurate).

According to Rubinow, previous studies did not have well-defined eligibility criteria. In many cases, the sample included only women who had visited a premenstrual clinic—which did not mean that these women necessarily had premenstrual syndrome. Samples typically did not include control groups or women who might have had menstrually-related disorders but had not visited a clinic, he says.

Another common research problem is "a bias among people to correlate distinct items—such as mood changes or headaches and menstruation—whether or not these items are, in fact, temporally related," he adds.

The critical question for the researcher, as he sees it, is to differentiate between mood changes triggered by menses and mood changes which might occur on a fairly regular basis without relation to menstruation.

"The researcher must therefore study these women over a long period of time before inferences and correlations between menses and mood changes can be accepted as fact."

While the definition of "premenstrual syndrome" has varied from study to study, Rubinow believes that the emphasis on "premenstrual" may obscure some potentially important relationships. Consequently, the women in his study are not bound by any arbitrary time definition as to when mood disorders related to menstruation occur.

If a mood disorder repeatedly and reliably occurs two days after the onset of menstruation, or every other month five days before the onset, the significance of these occurrences receives as much weight as a mood disorder occurring 10 days before menstruation, he indicates.

The women in Rubinow's research groups also are a homogeneous sample with respect to the strict DSM-III criteria for severe mood disorders. These criteria include apathy, impaired cognition, severe crying, sleep and appetite disorders, and either an inability to go to work or extreme difficulty in attending to one's work duties.

Rubinow's study started approximately a year ago and will continue for an indefinite time. Women in the study fill out various mood-rating scales, see researchers several times a month, and undergo a wide variety and long series of physiological tests.

As to whether men experience mood changes due to biological cycles, Rubinow speculates that they do. But this relationship is difficult to detect without an external cue, such as menstruation, he adds.

A study which compared normal men and women found that both sexes have similar cyclic mood changes. "Men are certainly susceptible to bipolar affective illnesses which are cyclic," Rubinow points out. "Looking at women and their menstrually-related mood changes might shed some light on cues to look for in men."

The NIMH scientist is enthusiastic about his research especially "because I see certain sets of symptoms clustering around different biological states. One sees different states rather than different symptoms."

For example, "women tell me that during certain times of the month, they misperceive colleagues' statements as personal attacks—yet they know, intellectually, that these remarks are innocuous."

They also report food cravings (9 out of 10 women crave carbohydrates) and a profound sense of guilt during a few days each month. The rest of the time, such food cravings, guilt feelings, and misperceptions disappear.

The psychiatrist hopes his research will clear up some of the stereotypes society has about menstrually-related disorders. "When a person has different feelings, and different sleep patterns, it doesn't make sense to view these symptoms without an overriding principle," he states. "This is a different, but integrated way of perceiving the world."

Women who think they might have menstrually-related mood disorders and are interesting in volunteering for the study may contact Dr. Rubinow at (301) 496-6825. ■

Medical Capsules

Sandra D. Levine

- A 5-year contract, coordinated by the National Institute of General Medical Sciences (NIGMS), has been awarded to create and maintain a new data bank on base molecules in nucleic acids.

In recent years, modern techniques for analyzing nucleic acids have led to an explosion of data concerning the composition of DNA and RNA molecules crucial to understanding complex genetic information. Readily available sequence information on base molecules in nucleic acids will help scientists, particularly geneticists and molecular biologists, build a foundation for further advances in biomedical research.

Cosponsors of the new data bank project are NIGMS, the National Institute of Allergy and Infectious Diseases, the National Cancer Institute, the Division of Research Resources, the National Science Foundation, the Department of Energy, and the Department of Defense.

- After a decade of research, the National Caries Program (NCP) of the National Institute of Dental Research (NIDR) has made considerable progress toward preventing tooth decay.

Since its inception in 1972, the NCP has been investigating four areas of research in caries prevention. Qne strategy involves combating the microorganisms (bacteria such as *Streptococcus mutans)* that induce decay. Another encompasses efforts to increase the resistance of teeth to decay through the expanded use of fluorides and plastic sealants. The other two areas of research include diet modification, e.g., substituting non-cariogenic natural and artificial sweeteners for sugar, and improving the delivery and acceptance of prevention methods.

Tooth decay remains a major health problem, but it is being reduced through prevention research and public education. A recent NCP survey indicated about a 30 percent decrease in tooth decay among U.S. school children in the last ten years.

- A technique called digital subtraction radiography, which couples digital computers to X-ray sensors to improve the quality of images, has been adapted for dental use.

Currently used to facilitate diagnosis in heart disease, the technique has been applied to hard tissue by radiologists at the National Institute of Dental Research (NIDR). The computer-processed images allow changes in bone to be detected that cannot be seen on conventional X-rays and enable artifacts to be screened out. Scientists are, therefore, exploring its potential for early diagnosis of periodontal and other dental diseases.

- Twenty years ago, Congress mandated the establishment of a separate NIH institute whose mission would be to support research and research training in the basic biomedical sciences.

The National Institute of General Medical Sciences (NIGMS) today funds a broad array of studies that seek new knowledge about how life processes work at their most basic cellular and molecular levels. This kind of research lays the essential groundwork for further clinical progress in the prevention, diagnosis, and treatment of disease.

With a current budget of about $335 million per year, NIGMS carries out its mission through five programs: Cellular and Molecular Basis of Disease, Genetics, Pharmacological Sciences, Physiology and Biomedical Engineering, and Minority Access to Research Careers (MARC). The first four fund both research and research training in their specific areas. The MARC program, a special training initiative, seeks to increase the numbers and capabilities of minority scientists engaged in biomedical research and to strengthen science curricula and research opportunities at institutions with significant minority enrollments.

Achievements of NIGMS-supported researchers in the field of genetics are helping scientists gain new knowledge about the growth and development of cells, tissues and organs; the regulation of gene activity; the evolution of species; and the bases of certain genetic diseases. NIGMS grantees have been leaders in the development of recombinant DNA technology and have also made major discoveries in such areas as the synthesis and transport of body proteins, the structure and function of cell membranes, the mechanisms of drug action in the body, and the body's response to severe trauma and burns.

- The National Cancer Institute (NCI) has developed a cancer treatment information database accessible through the MEDLARS System of the National Library of Medicine (NLM).

The database, known as Protocol Data Query (PDQ), contains over 600 research treatment plans currently in use, and the names of physicians conducting NCI-funded clinical trials at institutions throughout the country, cross-referenced by cancer site, treatment protocol and geographic location.

The first stage PDQ went into operation in October, and will be updated each month. At a future stage, PDQ will provide additional treatment information responsive to the needs of individual patients and physicians.

Further information on PDQ is available from NCI's Office of Cancer Communcations, 496-6641.

- About 10 percent of people over 40 years of age suffer from diverticulosis, a digestive disorder characterized by small sacs of intestinal lining which protrude along the wall of the large intestine. This figure jumps to almost 50 percent in people over 60. A small number also develop the complication of diverticulitis, or inflammation of these intestinal protrusions.

The Division of Digestive Diseases and Nutrition of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (NIADDK) is supporting research to investigate the causes, more effective treatment, and prevention of these conditions. A fact sheet on this disorder is available from the Office of Health Research Reports, NIADDK, Bldg. 31, Room 9A04, Bethesda, Md. 20205 (301-496-3583). Further information about digestive disorders is available from the Digestive Diseases Clearinghouse, 1555 Wilson Blvd., Suite 600, Rosslyn, VA, 22209, or phone 301-496-9707.

Editors of publications may request a glossy print of the numbered photographs appearing in this issue.

Ms. Maureen Napper
National Institutes of Health
Building, 31, Rm. 2B-10
Bethesda, Maryland 20205

Ms. Maureen Napper:

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